Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
S1	4	glnap$8	USPAT	OR	OFF	2004/02/16 14:59
S2	197050	acetyl phosphate	USPAT	OR	OFF	2000/09/27 11:43
S3	100	acetyl adj phosphate or acetylphosphate	USPAT	OR	OFF	2004/02/16 14:27
S4	33716	promoter	USPAT	OR	OFF	2004/02/16 14:28
S5	990133	induc$8 or regulat$8 or activat$8 or modulat$8	USPAT	OR	OFF	2004/02/16 14:29
S6	0	S3 same S4 same S5	USPAT	OR	OFF	2000/09/27 11:47
S7	42770	promoter$1	USPAT	OR	OFF	2004/02/16 14:28
S8	248634	acetate	USPAT	OR	OFF	2004/02/16 14:33
S9	274	(S3 or S8) same S7 same S5	USPAT	OR	OFF	2000/09/27 11:50
S10	44	(S3 or S8) near8 S5 same S7	USPAT	OR	OFF	2000/09/27 11:51
S12	7	glnap$8	US-PGPUB; USPAT	OR	OFF	2004/02/16 14:21
S13	219	acetyl adj phosphate or acetylphosphate	US-PGPUB; USPAT	OR	OFF	2004/02/16 14:27
S14	75283	promoter	US-PGPUB; USPAT	OR	OFF	2004/02/16 14:28
S15	90568	promoter$1	US-PGPUB; USPAT	OR	OFF	2004/02/16 14:28
S16	1535027	induc$8 or regulat$8 or activat$8 or modulat$8	US-PGPUB; USPAT	OR	OFF	2004/02/16 14:29
S17	1	S13 same S15 same S16	US-PGPUB; USPAT	OR	OFF	2004/02/16 14:34
S18	362722	acetate	US-PGPUB; USPAT	OR	OFF	2004/02/16 14:33
S19	341	S18 near8 S16 same S15	US-PGPUB; USPAT	OR	OFF	2004/02/16 14:36
S20	44	S18 near8 S16 near8 S15	US-PGPUB; USPAT	OR	OFF	2004/02/16 14:37
S21	6162	isoprenoid$1 or lycopene$1 or carotene$1 or astaxanthin$1 or phytoene$1 or isopentyl adj diphosphate or ipp	USPAT	OR	OFF	2004/02/16 15:36
S22	147	acetyl adj phosphate or acetylphosphate	USPAT	OR	OFF	2004/02/16 15:05
S23	219	acetyl adj phosphate or acetylphosphate	US-PGPUB; USPAT	OR	OFF	2004/02/16 15:05
S24	0	S21 near4 (gene$1 or sequence$1) and (S22 or S23)	USPAT	OR	OFF	2004/02/16 15:07
S25	306498	acetate	USPAT	OR	OFF	2004/02/16 15:06

S26	122	S21 near4 (gene$1 or sequence$1) and S25	US-PGPUB; USPAT	OR	OFF	2004/02/16 15:13
S27	0	S21 near4 (gene$1 or sequence$1) and S22	US-PGPUB; USPAT	OR	OFF	2004/02/16 15:22
S28	1278906	induc$8 or regulat$8 or activat$8 or modulat$8	USPAT	OR	OFF	2004/02/16 15:13
S29	12	S21 near4 (gene$1 or sequence$1) and (S25 same S28)	US-PGPUB; USPAT	OR	OFF	2004/02/16 15:13
S30	721	S21 same (S22 or S25)	US-PGPUB; USPAT	ADJ	OFF	2004/02/16 15:22
S31	2	S21 near4 (gene$1 or sequence$1) same (S22 or S25)	US-PGPUB; USPAT	OR	OFF	2004/02/16 15:24
S32	6	S26 and carbon adj (flux or flow)	US-PGPUB; USPAT	OR	OFF	2004/02/16 15:35
S33	62775	promoter$1	USPAT	OR	OFF	2004/02/16 15:35
S34	507	(S22 or S25) same S33 same S28	USPAT	OR	OFF	2004/02/16 15:35
S35	17	S21 and S34	US-PGPUB; USPAT	OR	OFF	2004/02/16 15:37

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	7	glnap$8	US-PGPUB; USPAT	OR	OFF	2004/02/16 14:21

US-PAT-NO: 6598188

DOCUMENT-IDENTIFIER: US 6598188 B1

TITLE: Error-corrected codeword configuration and method

DATE-ISSUED: July 22, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Locke; Michael	Santa Clara	CA	N/A	N/A
Gulati; Kapil	Sunnyvale	CA	N/A	N/A

APPL-NO: 09/ 569104

DATE FILED: May 10, 2000

PARENT-CASE:

RELATED APPLICATIONS

This application claims priority from provisional application serial No. 60/133,365, filed May 10, 1999.

US-CL-CURRENT: 714/704, 714/759 , 714/779

ABSTRACT:

Modem selection of Reed-Solomon codeword configuration to maximize error-corrected data rate given channel analysis. A lookup table of maximal codeword size given parity bytes and channel MSE allows precomputation.

1 Claims, 3 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 2

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Detailed Description Paragraph Table - DETL (1):

The tables parity_tbl[ ] [ ] and parity_trellis_tbl[ ] [ ] are generated in the Make-Table section following the codeword size method. Note that these tables are generated off line in order to simplify the rate adaptation algorithm and reduce the real time computational effort. // For the table without trellis coding for (each gap to try) [compute probability of constellation errors from Q function for (all possible number of parity bytes) [for (max to min codeword length) [if (error rate after RS corrections is acceptable) [record codeword length at parity_table[parity_bytes/2] [gap/ scale_factor]; cycle out of loop and test a different number of parity bytes]]]] // For the table with trellis coding for (each gap to try) [compute probability of constellation errors from tellis error rate estimation polynomial for (all possible number of parity bytes) [for (max to min codeword length) [if (error rate after RS corrections is acceptable) [record codeword length at parity_table[parity_bytes/2] [gap/ scale_factor]; cycle out of loop and test a different number of parity bytes]]]]

********** For udi_c.c, the algorithm for computing 4 optimized codeword configurations from parity_tbl[ ] [ ] or parity_trellis_tbl[ ] [ ] and some performance measurements. A number of data rate constraints are provided to this function that are not part of optimizing the rate.
****

Select a minimum number of parity bytes based on the user provided parameters (Not shown in the cut-down code) Select the correct parity table according to trellis decoding or not. The pointer "parity" is made to point to the correct table. Determine the latency path of the adaptive data channel. Note that this is very DMT-ADSL specific and not relevant to the generic preferred embodiments. However, it is needed to make sense of the code that follows. Compute the number of data bytes that can be sent over one data channel without any parity bytes. Note that the parameters provided as input assume that only one data channel is in use. The formula is Adjust max_bytes for the coding gain due to trellis coding. It might also be possible to adjust the coding gain for this factor instead. Adjust the data received from the ATUR as needed for high data rates. Note that the information is reported in a peculiar format, please refer to the T1.413 issue 2 standard. max_bits = input_bits-input_parity_bits-trellis_bits- coding_gain_bits estimate: coding_gain_bits = coding_gain*tones/3dB for (each option to optimize) Adjust max_bits for a certain amount of margin, according to the option number. Enforce minimum number of parity byte rules for each data latency. Initialize rate optimization loop variables maxbytes = maxbits/8; adaptive data latency bytes = maxbytes-non_adaptive data latency bytes - minumum required parity bytes if (trellis) gap = 9.8dB else gap = 5.3dB Setup best performance found to minimum data rate while (gap > the smallest gap that ever can work) if (host limits adaptive data rate below current) extra_bytes = byte capacity not allowed by the limitation else extra_bytes = -1 repeat until break For the non adaptive path compute frames per codeword (fpcw) and codewords per frame (cwpf) with the assumption that the largest codeword size is best. Compute the allowed interleave depth based on fpcw and input parameters. Enforce rules for limitations on fpcw and interleave depth according to the T1.413 standard. Estimate the burst error coding loss as loss = 1 if (not trellis & interleave depth is 1) loss = 2 if (trellis) interleave depth = 1 => loss = 8 interleave depth = 2 => loss = 4 interleave depth = 4 => loss = 2 if (parity or adaptive bytes available out of range) quit t = parity bytes per codeword/(loss*2) t is the effective correcting power of the proposed RS codeword if parity_table[gap] [t] < non_adaptive_codeword_length add a parity byte subtract a byte from the adaptive path else break out of loop END OF REPEAT UNTIL BREAK if (extra_bytes < 0) // some parity needed in adaptive path // Figure out the needed number of parity bytes for (t ranging from min to max) if (parity_table[gap] [t] >= adaptive_codeword_length) break out of loop else t = 0 if (t<=max) & (net adaptive data rate is higher than best encountered) record the current RS configuration as the best encountered configuration and data rate Increase maxbytes by 1 Decrease the gap accordingly gap = gap - 8bits/(tones*3dB) // Make gap computation numerically stable for fixed point // by changing the units and also computing // current_gap = start_gap - 8bits (currentbytes- startingbytes)/(tones*3dB) END OF WHILE GAP IN RANGE Re-enforce user supplied data maximums Record the optimum adaptive and non-adaptive RS configuration to CRatesRA string. END OF FOR EACH OPTION END OF FUNCTION

********** The following code determines the correct option to use from a group of 4 supplied options. This requires evaluating the performance of the supplied RS configurations and determining the highest data rate that matches the current conditions.
****

Determine the number of bytes needed for each latency path for each option Determine the correct parity_tbl to use, based on trellis decoding or not for each option // Compute gap for fast latency path followed by // computing gap for interleave latency path // Note trick used to compute correct correcting power. required gap = min gap if trellis enabled correcting power = (parity bytes per codeword)/16 else correcting power = (parity bytes per codeword)/4 for (each latency path) for (min to max coding gap) if (parity_tbl[correcting power] [gap] >= codeword size break out of loop if (min gap > required gap) required gap = min gap // Compute gap for interleaved latency path if trellis enabled if (interleave depth >= 8) correcting power = (parity bytes per codeword)/2 if (interleave depth == 4) correcting power = (parity bytes per codeword)/4 if (interleave depth == 2) correcting power = (parity bytes per codeword)/8 if (interleave depth == 1) correcting power = (parity bytes per codeword)/16 else if (interleave depth >= 2) correcting power = (parity bytes per codeword)/2 else correcting power = (parity bytes per codeword)/4 // END FOR EACH LATENCY PATH Call function to compute bit capacity at required gap Add in additional bits required for trellis if sufficient bit capacity break out of loop END FOR EACH OPTION return last tested option number Note that this will return option 4 if no option can be supported by the channel. Make Table generates parity_tbl[ ] [ ] and parity_trellis_tbl[ ] [ ]: #include "stdio.h" #include "stdlib.h" #include "math.h" #include "typedefs.h" // Gamma functions and erf taken from "Numerical Recipies" in Fortran // and translated to c double gammln (double xx) [state double cof[6]=[76.18009173, -86.50532033, 24.01409822, -1.231739516, 0.120858003e-2, -0.536382e-5]; static double stp = 2.50662827465; double x,tmp,ser; int i; x = xx-1.0; tmp = x+5.5; tmp = (x+0.5)*log(tmp)-tmp; ser = 1.0; for (i=0;i<6;i++) [x += 1.0; ser = ser+cof[i]/x;] return tmp+log(stp*ser);] double gser(double a, double x) [double gln,ap,sum,del; int i; gln = gammln(a); if (x==0) return 0; ap = a; sum = 1.0/a; del = sum; for (i=0;i<100;i++) [ap += 1.0; del *= x/ap; sum += del; if (fabs(del) < (fabs(sum)*3.0e-7)) break;] return sum*exp(-x*a*log(x)-gln);] double gcf(double a, double x) [double gln, gold, a0,a1,b0,b1,fac,an,ana,anf,g; int i; gln = gammln(a); gold = 0; a0 = 1.0; a1 = x; b0 = 0; b1 = 1; fac = 1.0; for (i=0;i<100;i++) [an = i+1; ana = an-a; a0 = (a1+a0*ana)*fac; b0 = (b1+b0*ana)*fac;

US-PAT-NO: 6254874

DOCUMENT-IDENTIFIER: US 6254874 B1

TITLE: Attenuated auxotrophic microorganisms having a combination of non-attenuating mutations and method for making same

DATE-ISSUED: July 3, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Mekalanos; John J.	Cambridge	MA	N/A	N/A
Klose; Karl E.	Newton	MA	N/A	N/A

APPL-NO: 08/ 421207

DATE FILED: April 13, 1995

US-CL-CURRENT: 424/234.1, 424/184.1 , 424/258.1 , 424/93.2 , 424/93.4 , 424/93.48 , 435/252.33 , 435/252.8 , 435/471 , 435/69.3

ABSTRACT:

A live non-virulent vaccine composition and method for preparing the same comprising a virulent microorganismal strain which contains at least two mutations, wherein the first mutation results in an auxotrophic mutant which requires for proliferation, a nutrient which is normally available in the host tissues in an amount required by the auxotrophic mutant for proliferation and the second mutation results in the inability of the auxotrophic mutant to specifically transport the required nutrient from host-tissues into the auxotrophic mutant thereby producing an attenuated strain.

57 Claims, 4 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 4

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Detailed Description Text - DETX (11):

Glutamine synthetase levels are modulated by controlling the level of transcription of glnA. glnA is a member of the glnA ntrBC operon, shown in FIG. 2, which includes in addition to glnA, two other nitrogen regulatory genes ntrB and ntrC, also referred to as glnL and glnG, respectively. Three promoters exist in the glnA ntrBC operon. glnAp1, with a transcriptional startsite located 187 base pairs (bp) upstream from the translational startsite in glnA; glnAp2, with a transcriptional startsite 85 bp upstream from the translational startsite in glnA; and ntrBp, with a transcriptional site located 256 bp downstream from the translational termination site in glnA and 33 bp upstream from the translational startsite in ntrB. Reitzer and Magasanik, Escherichia coli and Salmonella typhimurium, Cellular and Molecular Biology, American Society of Microbiology, pp. 302-320 (1987), herein incorporated by reference.

Detailed Description Text - DETX (12):
Under conditions of nitrogen excess, both NTRC and NTRB (also referred to as NR.sub.I and NR.sub.II, respectively) the nitrogen regulatory proteins of structural genes ntrC and ntrB, respectively, exist at low levels predominantly in their inactive forms. NTRC is a DNA binding protein that recognizes and binds two adjacent sequences (labeled 1 and 2, in FIG. 2) located within the glnAp1 promoter and one sequence (labeled 3) located within ntrBp. Transcription of glutamine synthetase, under nitrogen excess conditions, occurs at low levels through activation of glnAp1 by .sigma..sup.70 RNA polymerase.

Detailed Description Text - DETX (13):
RNA polymerase or the holoenzyme, as it is commonly referred to, can be separated biochemically into two components, the core enzyme and the sigma factor (the .sigma. polypeptide). The names reflect the fact that only the holoenzyme can initiate transcription; but then the sigma "factor" is released, leaving the core enzyme to undertake elongation. The function of the sigma factor is to ensure that RNA polymerase binds stably to DNA and initiates transcription only at promoters, not at other sites. Sigma factor also confers the ability to recognize specific binding sites and different sigma factors recognize different promoters. Therefore, .sigma..sup.70 is the sigma factor, discussed previously, responsible for recognizing the glnAp1 promoter and initiating transcription of glutamine synthetase.

Detailed Description Text - DETX (14):
When nitrogen becomes limiting, NTRB is activated in response to a lower level of internal glutamine such that it causes phosphorylation of NTRC; NTRC-phosphate then acts as a transcriptional activator, activating initiation of transcription at glnAp2 by .sigma..sup.54 -RNA polymerase (.sigma..sup.54 associated with core RNA polymerase). NTRC phosphate catalyzes the isomerization of the closed .sigma..sup.54 -RNA polymerase promoter complex to an open complex. Consequently, glutamine synthetase expression is initiated at the major glnA promoter, glnAp2, resulting in high levels of glutamine synthetase, NTRB and NTRC in order to increase internal glutamine levels. The resulting increase in the level of NTRC furthermore results in the complete repression of transcription initiating at glnAp1 and ntrBp. As discussed previously, NTRC binding sites exist within glnAp1 and ntrBp and as a result of the increasing levels of NTRC-phosphate bound at these sites .sigma..sup.70 RNA polymerase is prevented from recognizing and initiating transcription at the glnAp1 and ntrBp promoters. Shifting the microorganisms from nitrogen starvation to nitrogen excess causes NTRB to facilitate the removal of phosphate from NTRC-phosphate, bringing an end to the initiation of transcription at glnAp2. Continued growth in this nitrogen rich media results in the decline of levels of glutamine synthetase and NTRC by dilution. Consequently, the repression of glnAp1 and ntrBp by NTRC is lifted sufficiently to allow both glutamine synthetase and NTRC to be maintained at their respective low levels.

Detailed Description Text - DETX (15):
.sigma..sup.54 (also referred to as .sigma..sup.60, .sigma..sup.N, or NTRA) is encoded by the structural gene ntrA (alternately designated glnF or rpoN) and, as discussed above, is a positive regulatory factor needed for the expression of the gene encoding glutamine synthetase from the major glnAp2 promoters. .sigma..sup.54, is required for the transcription of the nitrogen fixation (nif) genes in a number of bacteria, and is required for transcription of genes encoding amino acid transport, such as glutamine, degradative enzymes,

as well as many other genes. See, Dixon, R., J. Gen. Microbiol., 130:2745-2755 (1984), Magasanik, B., Annu. Rev. Genet., 16:135-168 (1982), and S. Kustu et al., Micro. Biol. Reviews, 53:367-376 (1989).

Detailed Description Text - DETX (23):

In an alternate embodiment, live vaccines prepared in accordance with the preferred embodiment which have non-reverting mutations in genes under nitrogen control may be used as vectors or carriers for antigens of species other than the non-virulent pathogenic vaccine. One or more structural genes coding for the desired antigens may be operatively linked to an ntr-regulated promoter. As the non-virulent pathogenic microbe senses a nitrogen deficiency, expression of the ntr genes increases accordingly, thus initiating expression of genes transcribed from ntr promoters. For example, one or more structural genes coding for the desired antigens may be operatively linked to the glnAp2 promoter located on a suicide vector or an autonomously replicating plasmid. The suicide vector or plasmid may then be introduced into the non-virulent pathogenic strain having non-reverting mutations located in the genes glnA and glnH or glnQ, by well known methods, such as, transduction, transformation, electroporation, tri-parental mating techniques or direct transfer of a self mobilized vector in a bi-parental mating and maintained within the microorganism either autonomously or in the bacterial host's chromosomal DNA. Therefore, under low internal glutamine conditions which this attenuated strain must experience, the glnAp2 promoter will be activated and express the structural genes that have been operatively linked thereto. As discussed, in detail above, activation of an ntr promoter requires the proteins NTRA and NTRC, therefore this alternate embodiment will only work if the ntrA and ntrC genes are fully functional. Thus, this particular embodiment would be based on a strain prepared according to the preferred embodiment of the present invention and not the second embodiment of the present invention.

US-PAT-NO: 6162640

DOCUMENT-IDENTIFIER: US 6162640 A
See image for Certificate of Correction

TITLE: Selection methods

DATE-ISSUED: December 19, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Wohlstadter; Jacob Nathaniel	Andover	MA	01810	N/A

APPL-NO: 08/ 447515

DATE FILED: May 23, 1995

PARENT-CASE:

This application is a division of copending U.S. application Ser. No. 08/235,437, filed Apr. 29, 1994, which in turn is a continuation of U.S. application Ser. No. 07/852,412, filed Mar. 16, 1992 and now abandoned.

US-CL-CURRENT: 435/325, 435/243 , 435/440 , 435/6

ABSTRACT:

A rational method for obtaining a novel molecule capable of a desired interaction with a substrate of interest comprising selecting hosts or replicators which incode said novel molecules based upon cell or replicator growth caused by the desired interaction of the novel molecule and a selection molecule expressed by said host.

78 Claims, 9 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 9

---------- KWIC ---------

Detailed Description Text - DETX (285):
The cells are then incubated in glutamine and nitrogen limiting media. Cells capable of growth are selected. In this example selection occurs through a cascade. A properly phosphorylated NRI is capable of acting as an enhancer to the promoter glnApZ recognized by .sigma..sup.54 by binding an upstream DNA sequence. The enhanced glnApZ promoter then allows for the high level expression of glnA (glutamine synthetase) and DNaA. These proteins then in turn activate cascades which allow for cellular growth and replication.

US-PAT-NO: 6087177

DOCUMENT-IDENTIFIER: US 6087177 A

TITLE: Selection methods

DATE-ISSUED: July 11, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Wohlstadter; Jacob Nathaniel	Andover	MA	01810	N/A

APPL-NO: 08/ 235437

DATE FILED: April 29, 1994

PARENT-CASE:

This is a continuation of application Ser. No. 07/852,412, filed Mar. 16, 1992, which is incorporated by reference herein in its entirety now abandoned.

US-CL-CURRENT: 435/440, 435/320.1

ABSTRACT:

A rational method for obtaining a novel molecule capable of a desired interaction with a substrate of interest comprising selecting hosts or replicators which encode said novel molecules based upon cell or replicator growth caused by the desired interaction of the novel molecule and a selection molecule expressed by said host.

66 Claims, 9 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 9

---------- KWIC ---------

Detailed Description Text - DETX (286):

The cells are then incubated in glutamine and nitrogen limiting media. Cells capable of growth are selected. In this example selection occurs through a cascade. A properly phosphorylated NR.sub.I is capable of acting as an enhancer to the promoter glnApZ recognized by .sigma..sup.54 by binding an upstream DNA sequence. The enhanced glnApZ promoter then allows for the high level expression of glnA (glutamine synthetase) and DNaA. These proteins then in turn active cascades which allow for cellular growth and replication.

US-PAT-NO: 6066499

DOCUMENT-IDENTIFIER: US 6066499 A

TITLE: Selection methods

DATE-ISSUED: May 23, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Wohlstadter; Jacob Nathaniel	Andover	MA	01810	N/A

APPL-NO: 08/ 447506

DATE FILED: May 23, 1995

PARENT-CASE:

This application is a division of U.S. application Ser. No. 08/235,437, filed Apr. 29, 1994, which in turn is a continuation of U.S. application Ser. No. 07/852,412, filed Mar. 16, 1992 and now abandoned.

US-CL-CURRENT: 435/325, 435/243 , 435/440 , 435/6

ABSTRACT:

A rational method for obtaining a novel molecule capable of a desired interaction with a substrate of interest comprising selecting hosts or replicators which encode said novel molecules based upon cell or replicator growth caused by the desired interaction of the molecule and a selection molecule expressed by said host.

168 Claims, 9 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 9

---------- KWIC ---------

Detailed Description Text - DETX (309):

The cells are then incubated in glutamine and nitrogen limiting media. Cells capable of growth are selected. In this example selection occurs through a cascade. A properly phosphorylated NR.sub.I is capable of acting as an enhancer to the promoter glnApZ recognized by .sigma..sup.54 by binding an upstream DNA sequence. The enhanced glnApZ promoter then allows for the high level expression of glna (glutamine synthetase) and DNa. These proteins then in turn activate cascades which allow for cellular growth and replication.

US-PAT-NO: 6043093

DOCUMENT-IDENTIFIER: US 6043093 A

TITLE: Selection methods

DATE-ISSUED: March 28, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Wohlstadter; Jacob Nathaniel	Andover	MA	01810	N/A

APPL-NO: 08/ 485324

DATE FILED: June 7, 1995

PARENT-CASE:

This application is a continuation of copending U.S. application Ser. No. 08/235,437, filed Apr. 29, 1994, which in turn is a continuation of U.S. application Ser. No. 07/852,412, filed Mar. 16, 1992 and now abandoned.

US-CL-CURRENT: 435/440, 435/243 , 435/325 , 435/6

ABSTRACT:

A rational method for obtaining a novel molecule capable of a desired interaction with a substrate of interest comprising selecting hosts or replicators which encode said novel molecules based upon cell or replicator growth caused by the desired interaction of the novel molecule and a selection molecule expressed by said host.

137 Claims, 9 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 9

---------- KWIC ---------

Detailed Description Text - DETX (278):

The cells are then incubated in glutamine and nitrogen limiting media. Cells capable of growth are selected. In this example selection occurs through a cascade. A properly phosphorylated NR.sub.I is capable of acting as an enhancer to the promoter glnApZ recognized by .sigma..sup.54 by binding an upstream DNA sequence. The enhanced glnApZ promoter then allows for the high level expression of glnA (glutamine synthetase) and DNaA. These proteins then in turn activate cascades which allow for cellular growth and replication.

US-PAT-NO: 4970147

DOCUMENT-IDENTIFIER: US 4970147 A

TITLE: Oxygen regulatable gene expression

DATE-ISSUED: November 13, 1990

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Huala; Eva	Belmont	MA	N/A	N/A
Gu; Qing	Cambridge	MA	N/A	N/A
Albright; Lisa	Reading	MA	N/A	N/A
Ausubel; Frederick	Newton	MA	N/A	N/A

APPL-NO: 07/ 169488

DATE FILED: March 17, 1988

US-CL-CURRENT: 435/69.1, 435/252.3 , 435/320.1 , 435/476 , 435/488 , 435/71.1 , 435/91.41 , 536/23.2 , 536/24.1

ABSTRACT:

The invention discloses that, in the presence of NifA and NtrA, the expression of a desired DNA molecule which has been operably linked to an ntrA-RNA polymerase and NifA dependent promoter can be regulated by regulating the oxygen level of the culture medium. The invention relates to vectors which may be used in accordance with this discovery, and to methods for using such vectors.

11 Claims, 4 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 4

---------- KWIC ---------

Detailed Description Text - DETX (73):

The requirements for an upstream binding site at a particular promoter in vivo might vary depending upon the NtrA-polymerase, the transcriptional activator and the concentration of the activator. In vivo and in vitro experiments with NtrC show that by increasing its concentration one can compensate for deletion of its binding site upstream of the glnAP.sub.2 promoter (Austin, S., et al., Mol. Microb. 1:92-100 (1987)).

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	7	glnap$8	US-PGPUB; USPAT	OR	OFF	2004/02/16 14:21
L2	219	acetyl adj phosphate or acetylphosphate	US-PGPUB; USPAT	OR	OFF	2004/02/16 14:27
L3	75283	promoter	US-PGPUB; USPAT	OR	OFF	2004/02/16 14:28
L4	90568	promoter$1	US-PGPUB; USPAT	OR	OFF	2004/02/16 14:28
L5	1535027	induc$8 or regulat$8 or activat$8 or modulat$8	US-PGPUB; USPAT	OR	OFF	2004/02/16 14:29
L6	1	2 same 4 same 5	US-PGPUB; USPAT	OR	OFF	2004/02/16 14:29

US-PAT-NO: 6495356

DOCUMENT-IDENTIFIER: US 6495356 B1

TITLE: Beryllofluoride analogues of acyl phosphate polypeptides

DATE-ISSUED: December 17, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Dalai; Yan	Albany	CA	N/A	N/A
Kustu; Sydney	Berkeley	CA	N/A	N/A
Cho; Ho S.	San Francisco	CA	N/A	N/A

APPL-NO: 09/ 705233

DATE FILED: November 1, 2000

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application No. 60/168,431, Filed Nov. 30, 1999, which application is incorporated herein by reference.

US-CL-CURRENT: 435/194

ABSTRACT:

The present invention features methods and compositions for production of persistent acyl phosphate analogues (e.g., aspartyl phosphate analogues) using beryllofluoride (BeF.sub.x), as well as polypeptides comprising such an acyl phosphate analogue and antibodies that specifically bind to these polypeptides. The invention further features methods of using BeFx analogues in screening assays to identify candidate agent compounds that modulate activity of polypeptides that normally exhibit activity due to the presence of an acyl phosphate linkage (e.g., a phosphorylated aspartate residue as in, e.g., polypeptides involved in signal transduction, polypeptides involved in ion transport across biological membranes, phosphotransferases, etc.). The BeFx polypeptide analogues can also be used to facilitate determination of the structure of the corresponding phosphorylated polypeptide and in rationale drug design.

15 Claims, 19 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 13

---------- KWIC ---------

Detailed Description Text - DETX (82):

To determine whether effects of BeF.sub.x on NtrC could be generalized to other response regulators/receiver domains, we studied four additional proteins: E. coli OmpR and NarL, which activate and repress transcription by

.sigma.sup.70 -holoenzyme; E. coli CheY, which is a central regulator of the chemotactic response; and Bacillus subtilis Spo0F, which is part of the phosphorelay controlling initiation of sporulation. OmpR and NarL are two-domain response regulators. OmpR controls expression of outer membrane porin proteins in response to osmolarity and a variety of other signals that are poorly defined (Pratt et al. (1996) Mol. Microbiol. 20, 911-917; Egger et al. (1997) Genes To Cells 2, 167-184), whereas NarL controls gene expression in response to availability of the respiratory oxidants nitrate and nitrite (Stewart and Rabin (1995) in Two component signal transduction, eds. Hoch and Silhavy (ASM, Washington, D.C., USA), pp. 233-252). Although phosphorylation of OmpR affects its DNA-binding activity (Aiba et al. (1989) J. Biochem. 106, 5-7), it is not clear whether the N-terminal domain of OmpR acts positively or negatively. However, unlike the case for NtrC, the N-terminal receiver domain of NarL apparently acts negatively: structural studies provide evidence that the unphosphorylated receiver domain of NarL blocks DNA-binding by its C-terminal domain (Baikalov et al. (1996) Biochemistry 35, 11053-11061; Baikalov et al. (1998) Biochemistry 37, 3665-3676). Protections from cleavage by DNase I indicated that BeF.sub.x.OmpR bound to the regulatory region immediately upstream of the ompF promoter better than P-OmpR, whether phosphorylation was achieved with EnvZ (FIG. 4) or with acetyl phosphate (data not shown). Protections from DNase I cleavage and enhancements of cleavage indicated that BeF.sub.x.NarL and P-NarL bound similarly to the control region for the fdnG operon (data not shown), but under conditions optimal for the activation of NtrC (200 .mu.M BeCl.sub.2, 10 mM NaF and 5 mM MgCl.sub.2), BeF.sub.x.NarL was less active.

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	7	glnap$8	US-PGPUB; USPAT	OR	OFF	2004/02/16 14:21
L2	219	acetyl adj phosphate or acetylphosphate	US-PGPUB; USPAT	OR	OFF	2004/02/16 14:27
L3	75283	promoter	US-PGPUB; USPAT	OR	OFF	2004/02/16 14:28
L4	90568	promoter$1	US-PGPUB; USPAT	OR	OFF	2004/02/16 14:28
L5	1535027	induc$8 or regulat$8 or activat$8 or modulat$8	US-PGPUB; USPAT	OR	OFF	2004/02/16 14:29
L6	1	2 same 4 same 5	US-PGPUB; USPAT	OR	OFF	2004/02/16 14:34
L7	362722	acetate	US-PGPUB; USPAT	OR	OFF	2004/02/16 14:33
L8	341	7 near8 5 same 4	US-PGPUB; USPAT	OR	OFF	2004/02/16 14:36
L9	44	7 near8 5 near8 4	US-PGPUB; USPAT	OR	OFF	2004/02/16 14:37

PGPUB-DOCUMENT-NUMBER: 20030187294

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030187294 A1

TITLE: Process for producing catalysts comprising nanosize metal particles on a porous support, in particular for the gas-phase oxidation of ethylene and acetic acid to give vinyl acetate

PUBLICATION-DATE: October 2, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Hagemeyer, Alfred	Frankfurt		DE	
Dingerdissen, Uwe	Seeheim-Jugenheim		DE	
Kuhlein, Klaus	Kelkheim		DE	
Manz, Andreas	Sinzheim		DE	
Fischer, Roland	Neckargemund		DE	

APPL-NO: 10/ 395752

DATE FILED: March 24, 2003

RELATED-US-APPL-DATA:

child 10395752 A1 20030324

parent division-of 09485804 20000428 US GRANTED

parent-patent 6603038 US

child 09485804 20000428 US

parent a-371-of-international PCT/EP98/04819 19980801 WO UNKNOWN

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
DE	197 34 974.9	1997DE-197 34 974.9	August 13, 1997

US-CL-CURRENT: 560/241, 502/325 , 502/344

ABSTRACT:

The invention relates to a method for producing a catalyst containing one or several metals from the group of metals comprising the subgroups Ib and VIIIb of the periodic table on porous support particles, characterised by a first step in which one or several precursors from the group of compounds of metals from sub-groups Ib and VIIIb of the periodic table is or are applied to a porous support, and a second step in which the porous, preferably nanoporous support to which at least one precursor has been applied is treated with at least one reduction agent, to obtain the metal nanoparticles produced in situ in the pores of said support.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (131):

[0130] Some preferred catalyst systems which can be produced according to the invention, preferably coated catalysts, comprise, for example, not only palladium and gold but also potassium acetate as activator and/or cadmium or barium compounds as promoters.

PGPUB-DOCUMENT-NUMBER: 20030175775

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030175775 A1

TITLE: Ligand for G-protein coupled receptor GPR43 and uses thereof

PUBLICATION-DATE: September 18, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
LePoul, Emmanuel	Bruxelles		BE	
Detheux, Michel	Mons		BE	
Brezillon, Stephane	Dzlbeek		BE	
Lannoy, Vincent	Liernu		BE	
Parmentier, Marc	Beersel		BE	

APPL-NO: 10/ 337992

DATE FILED: January 7, 2003

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60346396 20020107 US

US-CL-CURRENT: 435/6, 435/7.1 , 514/1

ABSTRACT:

The present invention is related to the G-protein coupled orphan receptor GPR43 and the identification of short chain fatty acids as natural ligands of the receptor. The invention further relates to assays for the identification of agents that modulate GPR43 ligand binding and signalling activity, as well as compositions consisting essentially of an isolated GPR43 polypeptide and an isolated short chain fatty acid. The invention also relates to diagnostic methods and kits that take advantage of the novel interaction of GPR43 with short chain fatty acids.

PRIORITY

[0001] This application claims priority under 35 U.S.C. .sctn.119(e) to U.S. Provisional Application No. 60/346,396, filed Jan. 7, 2002.

---------- KWIC ---------

Detail Description Paragraph - DETX (108):

[0241] Additional examples of transcriptional control elements that are responsive to changes in GPCR activity include, but are not limited to those responsive to the AP-1 transcription factor and those responsive to NF-.kappa.B activity. The consensus AP-1 binding site is the palindrome TGA(C/G)TCA (Lee et al., 1987, Nature 325: 368-372; Lee et al., 1987, Cell 49: 741-752). The AP-1 site is also responsible for mediating induction by tumor promoters such as the phorbol ester 12-O-tetradecanoylphorbol- -acetate (TPA), and are therefore sometimes also referred to as a TRE, for TPA-response element. AP-1 activates numerous genes that are involved in the early response of cells to

growth stimuli. Examples of AP-1-responsive genes include, but are not limited to the genes for Fos and Jun (which proteins themselves make up AP-1 activity), Fos-related antigens (Fra) 1 and 2, I.kappa.B.alpha., ornithine decarboxylase, and annexins I and II.

PGPUB-DOCUMENT-NUMBER: 20030170856

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030170856 A1

TITLE: Regulation of human map kinase phosphatase-like enzyme

PUBLICATION-DATE: September 11, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Liou, Jiing-Ren	Belmont	MA	US	

APPL-NO: 10/ 363676

DATE FILED: April 24, 2003

PCT-DATA:
APPL-NO: PCT/EP01/09848
DATE-FILED: Aug 27, 2001
PUB-NO:
PUB-DATE:
371-DATE:
102(E)-DATE:

US-CL-CURRENT: 435/196, 435/194 , 435/320.1 , 435/325 , 435/6 , 435/69.1 , 536/23.2

ABSTRACT:

Reagents which regulate human MAP kinase phosphatase-like enzyme and reagents which bind to human MAP kinase phosphatase-like enzyme gene products can play a role in preventing, ameliorating, or correcting dysfunctions or diseases including, but not limited to, allergies including asthma, CNS disorders, diabetes, obesity, chronic obstructive pulmonary disease, cancer, and cardiovascular diseases.

---------- KWIC ---------

Detail Description Paragraph - DETX (295):

[0348] ERK activity is reduced in MAP kinase phosphatase-like enzyme overexpressing cells. Growth factor, hormone, and phorbol ester stimulation of PC12 cells have been known to activate the MAP kinase pathway and to stimulate the enzymatic activity of ERK-1 (61, 62, 65, 69, 71). The enzymatic activity of ERK-1 in wild type cells is compared with that in MAP kinase phosphatase-like enzyme overexpressing cells treated with these agents. PC12 cells treated for 10 minutes with mitogenic agents such as EGF, differentiating agents such as NGF and forskolin, and the tumor promoter phorbol 12-myristate 132-acetate (PMA), produce a robust activation of ERK-1 as measured by an immune complex activity assay. MAP kinase phosphatase-like enzyme overexpressing clones are then treated with the same agents for the identical times. These clones show a dramatic reduction in the ability of growth factors and hormones to activate ERK-1. Quantitation of the immune complex assays shows that modest overexpression of MAP kinase phosphatase-like enzyme in PC12 cells inhibits growth factor- and hormone-induced activation of ERKs 80-90%

cells compared to the fold activation seen in wild type PC12 cells. The basal ERK activity also appears to be lower in these overexpression cells as compared to wild type cells.

PGPUB-DOCUMENT-NUMBER: 20030104356

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030104356 A1

TITLE: Compounds and methods for treating and screening viral reactivation

PUBLICATION-DATE: June 5, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Berger, Shelly L.	Wayne	PA	US	

APPL-NO: 10/ 108164

DATE FILED: March 26, 2002

RELATED-US-APPL-DATA:

child 10108164 A1 20020326

parent continuation-of 09424348 19991122 US ABANDONED

US-CL-CURRENT: 435/5, 424/186.1

ABSTRACT:

This invention relates to host cellular factors as therapeutic and diagnostic compounds, and methods using such factors for screening for antiviral compounds, particularly compounds useful to treat Herpesvirus infections, such as HSV-1 and HSV-2 infections.

---------- KWIC ---------

Detail Description Paragraph - DETX (309):

[0287] Varnum, B. C., Lim, R. W. & Herschman, H. R. (1989). Characterization of TIS7, a gene induced in Swiss 3T3 cells by the tumor promoter tetradecanoyl phorbol acetate. Oncogene 4, 1263-1265.

PGPUB-DOCUMENT-NUMBER: 20030096833

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030096833 A1

TITLE: Substituted ideno[1,2-c]isoquinoline derivatives and methods of use thereof

PUBLICATION-DATE: May 22, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Jagtap, Prakash G.	Beverly	MA	US	
Baloglu, Erkan	Boston	MA	US	
van Duzer, John H.	Georgetown	MA	US	
Szabo, Csaba	Gloucester	MA	US	
Salzman, Andrew L.	Belmont	MA	US	

APPL-NO: 09/ 944524

DATE FILED: August 31, 2001

US-CL-CURRENT: 514/285, 546/62 , 546/70

ABSTRACT:

The invention provides a novel class of substituted indeno[1,2-c]isoquinoline derivatives. Pharmaceutical compositions and methods of making and using the compounds, are also described.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (57):

[0053] The compositions may be sterilized and/or contain minor amounts of non-toxic adjuvants, such as preserving, stabilizing, wetting or emulsifying agents, solution promoters, salts for regulating the osmotic pressure pH buffering agents, and other substances such as for example, sodium acetate, triethanolamine oleate, etc. In addition, they may also contain other therapeutically valuable substances.

PGPUB-DOCUMENT-NUMBER: 20030078212

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030078212 A1

TITLE: PHARMACEUTICAL COMPOSITIONS CONTAINING POLY(ADP-RIBOSE) GLYCOHYDROLASE INHIBITORS AND METHODS OF USING THE SAME

PUBLICATION-DATE: April 24, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
LI, JIA-HE	COCKEYSVILLE	MD	US	
ZHANG, JIE	ELLICOTT CITY	MD	US	

APPL-NO: 09/ 182645

DATE FILED: October 30, 1998

CONTINUED PROSECUTION APPLICATION: This is a publication of a continued prosecution application (CPA) filed under 37 CFR 1.53(d).

US-CL-CURRENT: 514/22, 514/25

ABSTRACT:

The present invention relates to pharmaceutical compositions containing poly(ADP-ribose) glucohydrolase inhibitors, also known as PARG inhibitors, and methods of using the same for inhibiting or decreasing free radical induced cellular energy depletion, cell damage, or cell death. More particularly, the present invention relates to pharmaceutical compositions containing poly (ADP-ribose) glucohydrolase inhibitors such as glucose derivatives; lignin glycosides; hydrolysable tannins including gallotannins and ellagitannins; adenoside derivatives; acridine derivatives including 6,9-diamino-2-ethoxyacridine lactate monohydrate; tilorone analogs including tilorone R10.556, daunomycin or daunorubicin hydrochloride; ellipticine; proflavine; and other PARG inhibitors; and their method of use in treating or preventing diseases or conditions due to free radical induced cellular energy depletion and/or tissue damage resulting from cell damage or death due to necrosis, apoptosis, or combinations thereof.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (29):

[0028] The use of the PARG inhibitor tannic acid for treating HIV infection is discussed in Uchiumi et al., "Inhibitory Effect of Tannic Acid on Human Immunodeficiency Virus Promoter Activity Induced by 12-O-Tetra Decanoylphorbol-13-acetate in Jurkat T-Cells", Biochem. Biophys. Res. Comm. 220:411-417 (1996).

PGPUB-DOCUMENT-NUMBER: 20020192262

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020192262 A1

TITLE: 1'-Acetoxychavicol acetate for tuberculosis treatment

PUBLICATION-DATE: December 19, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Palittapongarnpim, Prasit	Bangkok		TH	
Kirdmanee, Chalermpol	Bangkok		TH	
Kittakoop, Prasat	Prathumthani		TH	
Rukseree, Kamolchanok	Bangkok		TH	

APPL-NO: 10/ 152570

DATE FILED: May 23, 2002

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
TH	066318	2001TH-066318	June 18, 2001

US-CL-CURRENT: 424/422

ABSTRACT:

1'-Acetoxychavicol acetate is a compound not known before to possess anti-tuberculous activity. The above data revealed that the compound was active against the standard H37Ra strain as well as several clinical isolates at the concentration well below the toxic concentration against various mammalian cells. The compound is therefore potentially useful as an therapeutic and preventive agent for tuberculosis as well as an antiseptic agent against the bacteria.

---------- KWIC ---------

Detail Description Paragraph - DETX (31):

[0046] 8. Kondo, A., Ohigashi, H., Murakami, A., Suratwadee, J., and Koshimizu, K. 1'-Acetoxychavicol acetate as a potent inhibitor of tumor-promoter-induced Epstein-Barr virus activation from Languas galanga, a traditional Thai condiment. Biosci Biotechnol Biochem 1993;57:1344-1345.

US-PAT-NO: 6683050

DOCUMENT-IDENTIFIER: US 6683050 B1

TITLE: Compounds with anti-KS and anti-HIV activity

DATE-ISSUED: January 27, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Antakly; Tony	Montreal	N/A	N/A	CA
Sairam; Ram M.	Dollard-des-Ormeaux	N/A	N/A	CA

APPL-NO: 09/ 494500

DATE FILED: January 31, 2000

PARENT-CASE:

This application is a continuation of PCT/CA98/00731 filed Jul. 30, 1998 designating the United States and claiming priority of U.S. Provisional Patent Application Ser. No. 60/054,543 filed Aug. 1, 1997 (now abandoned).

US-CL-CURRENT: 514/2, 514/21 , 514/8 , 530/326 , 530/834

ABSTRACT:

The present invention relates to a compound having anti-KS and anti-HIV pharmaceutical activity which comprises an HCG-like inhibitory protein and fragments or derivatives thereof, said protein and fragments thereof are isolated from a biologically active fraction of APL-HCG, wherein said protein has a molecular weight of about 3,500 or of about 13,000 Dalton, and wherein said protein and fragments thereof are adsorbed polypropylene plastic supports. A pharmaceutical composition for the prevention and/or treatment of Kaposi's sarcoma (KS) and HIV which comprises an therapeutically effective amount of at least one compound of the present invention in association with a pharmaceutically acceptable carrier. A method for the prevention, treatment and/or reduction of Kaposi's sarcoma and HIV expression in AIDS patients, which consists in administering the composition to the patient.

4 Claims, 15 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 10

---------- KWIC ---------

Detailed Description Text - DETX (28):

Activating protein-1 (AP-1) is a transcriptional activator which is induced by 12-O-tetradecanyl phorbol-13-acetate (TPA) tumor promoter, several growth factors and various extracellular stimuli (reviewed in Saatcioglu F et al., 1994, Semin. Cancer Biol. 5:347-359). AP-1 consists of proteins of jun and fos families which associate to form homo-(jun/jun) or heterodimers (jun/fos) and recognize a consensus sequence 5'-TGA G/C TCA-3' known as TPA Response Element (TRE) present on AP-1 regulated genes. AP-1 complexes are considered

to play important roles in several signal transduction pathways such as growth stimulation, differentiation, neuronal excitation and transformation (Saatcioglu F et al., 1994, Semin. Cancer Biol. 5:347-359). APL-HCG and components in fraction 7 significantly inhibited AP-1 binding to TRE in KSY-1 cells (FIG. 2D). APL-HCG inhibited AP-1 binding by 1.5, 3 and 2 fold respectively after 3, 6 and 12 hours of treatment (FIG. 3).

US-PAT-NO: 6610516

DOCUMENT-IDENTIFIER: US 6610516 B1

TITLE: Cell culture process

DATE-ISSUED: August 26, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Andersen; Dana C.	Redwood City	CA	N/A	N/A
Bridges; Tiffany M.	Burlingame	CA	N/A	N/A
Gawlitzek; Martin	Foster City	CA	N/A	N/A
Hoy; Cynthia A.	Hillsborough	CA	N/A	N/A

APPL-NO: 09/ 723545

DATE FILED: November 27, 2000

PARENT-CASE:

This is a divisional application claiming priority to application Ser. No. 09/553,924, filed Apr. 21, 2000, which claims priority to U.S. Provisional Application Serial No. 60/131,076, filed Apr. 26, 1999, the entire disclosure of which is hereby incorporated by reference.

US-CL-CURRENT: 435/70.1, 435/252.3 , 435/358 , 435/69.1 , 530/395

ABSTRACT:

A glycoprotein is produced by a process comprising culturing mammalian host cells expressing nucleic acid encoding a glycoprotein in the presence of (a) a factor that modifies growth state in a cell culture, (b) a divalent metal cation that can adopt and prefers an octahedral coordination geometry, and/or (c) a plasma component. In this process, the occupancy of an N-linked glycosylation site occupied only in a fraction of a glycoprotein is enhanced. Such culturing is preferably carried out at a temperature of between about 30.degree. C. and 35.degree. C. and/or in the presence of up to about 2 mM of a butyrate salt and/or in the presence of a cell-cycle inhibitor.

1 Claims, 16 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 8

---------- KWIC ---------

Detailed Description Text - DETX (16):

By "plasma component" is meant a constituent of normal plasma. This would include growth promoters and tumor-promoting agents for endothelial cell growth, regulators of differentiation of epithelial tissues, glucagon, heparin, phorbol myristate acetate, PRL, thyroglobulin, 8Br-cAMP, thrombin, vitamin A and its derivatives (retinoids such as retinoic acid, e.g., beta-all-trans retinoic acid), glutathione, steroids such as corticosterone, cortisol, and corticoids, e.g., glucocorticoids such as hydrocortisone, and hormones,

preferably those that are vital hormones of metabolism such as estrogen, insulin, and thyroid hormones, e.g., thyroxine and tri-iodothyronine (T.sub.3). The thyroid hormones are preferred, and most preferably thyroxine and tri-iodothyronine. Since some serum, including fetal calf serum, contains thyroid hormones and the thyroid hormone binding protein at nanomolar levels, it is preferred to use serum-free medium, particularly if thyroid hormones are employed to enhance site-occupancy.

US-PAT-NO: 6603038

DOCUMENT-IDENTIFIER: US 6603038 B1

TITLE: Method for producing catalysts containing metal nanoparticles on a porous support, especially for gas phase oxidation of ethylene and acetic acid to form vinyl acetate

DATE-ISSUED: August 5, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Hagemeyer; Alfred	Frankfurt	N/A	N/A	DE
Dingerdissen; Uwe	Seeheim-Jugenheim	N/A	N/A	DE
Kuhlein; Klaus	Kelkheim	N/A	N/A	DE
Manz; Andreas	Sinzheim	N/A	N/A	DE
Fischer; Roland	Neckargemund	N/A	N/A	DE

APPL-NO: 09/ 485804

DATE FILED: April 28, 2000

PARENT-CASE:

This application is a 371 of PCT/EP98/04819 filed Aug. 1, 1998.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
DE	197 34 974	August 13, 1997

PCT-DATA:
APPL-NO: PCT/EP98/04819
DATE-FILED: August 1, 1998
PUB-NO: WO99/08791
PUB-DATE: Feb 25, 1999
371-DATE:
102(E)-DATE:

US-CL-CURRENT: 560/241.1, 502/325 , 502/330

ABSTRACT:

The invention relates to a method for producing a catalyst containing one or several metals from the group of metals comprising the sub-groups Ib and VIIIb of the periodic table on porous support particles, characterized by a first step in which one or several precursors from the group of compounds of metals from sub-groups Ib and VIIIb of the periodic table is or are applied to a porous support, and a second step in which the porous, preferably nanoporous support to which at least one precursor has been applied is treated with at least one reduction agent, to obtain the metal nanoparticles produced in situ in the pores of said support.

27 Claims, 0 Drawing figures

Exemplary Claim Number: 1

---------- KWIC ---------

Brief Summary Text - BSTX (113):

Some preferred catalyst systems which can be produced according to the invention, preferably coated catalysts, comprise, for example, not only palladium and gold but also potassium acetate as activator and/or cadmium or barium compounds as promoters.

US-PAT-NO: 6506598

DOCUMENT-IDENTIFIER: US 6506598 B1
See image for Certificate of Correction

TITLE: Cell culture process

DATE-ISSUED: January 14, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Andersen; Dana C.	Redwood City	CA	N/A	N/A
Bridges; Tiffany M.	Burlingame	CA	N/A	N/A
Gawlitzek; Martin	Foster City	CA	N/A	N/A
Hoy; Cynthia A.	Hillsborough	CA	N/A	N/A

APPL-NO: 09/ 553924

DATE FILED: April 21, 2000

PARENT-CASE:

This is a non-provisional application claiming priority to provisional application no. 60/131,076 , filed Apr. 26, 1999, the entire disclosure of which is hereby incorporated by reference.

US-CL-CURRENT: 435/359, 435/252.3 , 435/358 , 435/69.6 , 530/395

ABSTRACT:

A glycoprotein is produced by a process comprising culturing mammalian host cells expressing nucleic acid encoding said glycoprotein in the presence of (a) a factor that modifies growth state in a cell culture, (b) a divalent metal cation that can adopt and prefers an octahedral coordination geometry, and/or (c) a plasma component. In this process, the occupancy of an N-linked glycosylation site occupied only in a fraction of a glycoprotein is enhanced. Such culturing is preferably carried out at a temperature of between about 30.degree. C. and 35.degree. C. and/or in the presence of up to about 2 mM of a butyrate salt and/or in the presence of a cell-cycle inhibitor.

23 Claims, 16 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 8

---------- KWIC ---------

Detailed Description Text - DETX (14):
By "plasma component" is meant a constituent of normal plasma. This would include growth promoters and tumor-promoting agents for endothelial cell growth, regulators of differentiation of epithelial tissues, glucagon, heparin, phorbol myristate acetate, PRL, thyroglobulin, 8Br-cAMP, thrombin, vitamin A and its derivatives (retinoids such as retinoic acid, e.g., beta-all-trans retinoic acid), glutathione, steroids such as corticosterone, cortisol, and corticoids, e.g., glucocorticoids such as hydrocortisone, and hormones,

preferably those that are vital hormones of metabolism such as estrogen, insulin, and thyroid hormones, e.g., thyroxine and tri-iodothyronine (T.sub.3). The thyroid hormones are preferred, and most preferably thyroxine and tri-iodothyronine. Since some serum, including fetal calf serum, contains thyroid hormones and the thyroid hormone binding protein at nanomolar levels, it is preferred to use serum-free medium, particularly if thyroid hormones are employed to enhance site-occupancy.

US-PAT-NO: 6504048

DOCUMENT-IDENTIFIER: US 6504048 B1
See image for Certificate of Correction

TITLE: Flavorant compositions

DATE-ISSUED: January 7, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Bachmann; Jean-Pierre	Wadenswil	N/A	N/A	CH
Gautschi; Markus	Zeiningen	N/A	N/A	CH
Hostettler; Bernhard	Gockhausen	N/A	N/A	CH
Yang; Xiaogen	West Chester	OH	N/A	N/A

APPL-NO: 09/ 634029

DATE FILED: August 8, 2000

PARENT-CASE:

This is a Continuation Application of U.S. application Ser. No. 09/212,985, now Pat. No. 6,203,839 filed Dec. 16, 1998, now Pat. No. 6,203,839 which is incorporated herein by reference in its entirety. This application is also related to application Ser. No. 09/634,067, filed on even date herewith and entitled "Flavorant Compositions", and is incorporated herein by reference in its entirety.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
EP	97122633	December 22, 1997

US-CL-CURRENT: 560/254, 426/533 , 426/538 , 426/546 , 560/221

ABSTRACT:

The invention is related to a flavorant composition containing 1'-acetoxychavicol acetate and 1'-acetoxyeugenol acetate derivatives thereof as well as a flavorant acceptable carrier. The flavorant composition may be used for flavoring foods, beverages or healthcare products with warm/hot, spicy and pungent sensations related to Galangal.

15 Claims, 0 Drawing figures

Exemplary Claim Number: 1

---------- KWIC ---------

Other Reference Publication - OREF (8):
Kondo, et al., 1'Acetoxychavicol Acetate as a Potent Inhibitor of Tumor Promoter-induced Epstein-Barr Virus Activation from Lanquas galanga, a Traditional Thai Condiment, Biosci. Biotech. Biochem. 57(8) (1993) 1344-1345.

US-PAT-NO: 6476252

DOCUMENT-IDENTIFIER: US 6476252 B1
See image for Certificate of Correction

TITLE: Flavorant compositions

DATE-ISSUED: November 5, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Bachmann; Jean-Pierre	Wadenswil	N/A	N/A	CH
Gautschi; Markus	Zeiningen	N/A	N/A	CH
Hostettler; Bernhard	Gockhausen	N/A	N/A	CH
Yang; Xiaogen	West Chester	OH	N/A	N/A

APPL-NO: 09/ 634067

DATE FILED: August 8, 2000

PARENT-CASE:

This is a Continuation Application of U.S. application Ser. No. 09/212,985, filed Dec. 16, 1998, which is incorporated herein by reference in its entirety. This application is also related to application Ser. No. 09/634,029, filed on even date herewith and entitled "Flavorant Compositions", and is incorporated herein by reference in it entirety.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
EP	97122633	December 22, 1997

US-CL-CURRENT: 560/130

ABSTRACT:

The invention is related to a flavorant composition containing 1'-acetoxychavicol acetate and 1'-acetoxyeugenol acetate derivatives thereof as well as a flavorant acceptable carrier. The flavorant composition may be used for flavoring foods, beverages or healthcare products with warm/hot, spicy and pungent sensations related to Galangal.

1 Claims, 0 Drawing figures

Exemplary Claim Number: 1

---------- KWIC ---------

Other Reference Publication - OREF (7):
Kondo, et al., 1'Acetoxychavicol Acetate as a Potent Inhibitor of Tumor Promoter-induced Epstein-Barr Virus Activation from Languas galanga, a Traditional Thai Condiment, Biosci, Biotech. Biochem. 57(8) (1993) 1344-1345.

US-PAT-NO: 6313373

DOCUMENT-IDENTIFIER: US 6313373 B1

TITLE: Tissue specific promoters and transgenic mouse for the screening of pharmaceuticals

DATE-ISSUED: November 6, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Eckert; Richard L.	Cleveland Hts.	OH	N/A	N/A
Crish; James F.	North Olmsted	OH	N/A	N/A

APPL-NO: 09/ 430201

DATE FILED: October 29, 1999

PARENT-CASE:

This application for a nonprovisional U.S. Utility Patent Application claims priority to Provisional U.S. patent application Ser. No. 60/106,495 filed on Oct. 30 1998.

US-CL-CURRENT: 800/18, 435/320.1 , 435/325 , 435/455 , 536/23.1 , 800/10 , 800/25 , 800/3

ABSTRACT:

The present invention provides human involucrin (hINV) sequences having tissue specific and cell type specific promoter activity. The sequences provided herein direct expression to suprabasal cells of stratifying epithelia. The invention further provides methods for the production of transgenic animals which contain a hINV promoter sequence which directs the expression of human papillomavirus 16 oncogenes (or other oncogenes). These animals display cervical and epidermal hyperplasias as well as cancer of the trachea, esophagus, colon, epidermis, anus/rectum, lymph nodes, spleen and lung. The animals of the invention provide a useful model for screening potential anti-neoplastic compounds, carcinogens, and co-carcinogens for a number of cancers.

28 Claims, 13 Drawing figures

Exemplary Claim Number: 15,20,21

Number of Drawing Sheets: 15

---------- KWIC ---------

Detailed Description Text - DETX (93):

DNA sequence analysis indicates that the DRR contains binding sites for several transcription factors, in including functionally important Sp1 and AP1 sites (Welter, et al. "Fos-related antigen (Fra-1), junB, and junD activate human involucrin promoter transcription by binding to proximal and distal AP1 sites to mediate phorbol ester effects on promoter activity" J. Biol. Chem. 270:12614-12622, 1995; Banks, et al. " Characterization of human involucrin

promoter distal regulatory region transcriptional activator elements-a role for Sp1 and AP1 binding sites" Biochem. J. 331:61-68, 1998; Efimova, et al. "Regulation of human involucrin promoter activity by a protein kinase C, Ras, MEKK1, MEK3, p38/RK, AP1 signal transduction pathway" J. Biol. Chem. 272:24387-24395, 1998). Our previous in vitro studies show that the basal promoter does not drive expression in karatinocytes; however, addition of the DRR restores hINV promoter activity (Banks, et al. "Characterization of human involucrin promoter distal regulatory region transcriptional activator elements-a role for Sp1 and AP1 binding sites" Biochem. J. 331:61-68, 1998). Moreover, mutation of the AP1 site (AP1-5) inactivates the promoter (see, e.g., Examples 7-9). AP1 has been shown to be an important regulator in several genes that are expressed in a differentiation-dependent manner in surface epithelia (DiSepio, et al. "The proximal promoter of the mouse loricrin gene contains a functional AP1 element and directs keratinocyte-specific but not differentiation-specific expression" J. Biol. Chem. 270:10792-10799, 1995; Takahashi and Iizuka "Analysis of the 5'-upstream promoter region of human involucrin gene: activation by 12-O-tetradecanoylphorbol-13-acetate" J. Invest. Dermatol. 100:10-15, 1993). With out limiting the present invention to any mechanism, the present investigation shows that the AP1 site contained within the nucleotide sequence from -2473 to -1953 of FIG. 6 is important in the regulation of expression of the hINV gene as well as any sequences that may be operably linked to said sequence.

US-PAT-NO: 6313165

DOCUMENT-IDENTIFIER: US 6313165 B1

TITLE: Inhibition of cataracts and other disorders

DATE-ISSUED: November 6, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Grunberger; Dezider	Teaneck	NJ	N/A	N/A
Frenkel; Krystyna	Woodmer	NY	N/A	N/A

APPL-NO: 08/ 704545

DATE FILED: December 23, 1996

PARENT-CASE:

This application is a 321 of PCT/US95/03392 filed Mar. 14, 1995, which is a CIP of Ser. No. 08/212,569 filed Mar. 14, 1994 now is U.S. Pat. No. 5,591,773.

PCT-DATA:
APPL-NO: PCT/US95/03392
DATE-FILED: March 14, 1995
PUB-NO:
PUB-DATE:
371-DATE: Dec 23, 1996
102(E)-DATE:Dec 23, 1996

US-CL-CURRENT: 514/532, 514/544

ABSTRACT:

A method of inhibiting the formation of a cataract in an eye by contacting the eye with a compound having the structure: ##STR1##

is described. Also described is a method of inhibiting the progression of cataract formation in an eye. Methods comprising administering a pharmaceutical composition comprising the above compound to inhibit the formation of a cataract in the eye of a subject and to inhibit progression of cataract formation in the eye of a subject are also described. The above compound also prevents diseases resulting from oxidative stress, including diseases comprising tumor formation resulting from oxidative stress, and also inhibits the progression of diseases resulting from oxidative stress. The above compound may furthermore be used to treat an HIV infection when combined in a pharmaceutical composition with a substance which inhibits HIV replication.

12 Claims, 13 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 13

---------- KWIC ---------

Brief Summary Text - BSTX (5):

It has recently been shown that the phorbol ester-type tumor promoters (12-O-tetradecanoylphorbol-13-acetate, hereinafter TPA) induce H.sub.2 O.sub.2 production in mouse skin as well as cause oxidation of DNA bases in vivo (20-22). In addition, it has been found that agents possessing anti-tumor-promoting properties in vivo, also suppress inflammatory processes. Processes suppressed by such agents include infiltration of polymorphonuclear leukocytes (hereinafter PMNs), reactive oxygen species production, and oxidation of DNA bases (20-22), as well as induction of ornithine decarboxylase (ODC) and edema (23-26). A number of known anti-tumor promoters that possess all or some of those properties have been isolated from biological sources, and include sarcophytol A (isolate from marine soft coral) (27, 28), (-)-epigallocatechin gallate (EGCG, a polyphenol from green tea) (26, 29, 30), curcumin (a spice) (24, 25), and caffeic acid (24, 25).

US-PAT-NO: 6312697

DOCUMENT-IDENTIFIER: US 6312697 B1

TITLE: Inhibitory effect of synthetic and natural colorants on carcinogenesis

DATE-ISSUED: November 6, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Kapadia; Govind J.	Potomac	MD	20854	N/A
Tokuda; Harukuni	N/A	N/A	N/A	N/A
N/A	Kyoto 602	N/A	N/A	JP
Konoshima; Takao	N/A	N/A	N/A	N/A
N/A	Kyoto 607	N/A	N/A	JP
Takasaki; Midori	Kyoto 602			JP
Nishino; Hoyoku	Kyoto 602			JP

APPL-NO: 09/ 256206

DATE FILED: February 24, 1999

PARENT-CASE:

This application is a divisional application of U.S. application Ser. No. 08/845,166, filed Apr. 21, 1997, which in turn claims the benefit of U.S. Provisional Application No. 60/022,638, filed Jul. 24, 1996. [, and of U.S. application Ser. No. 08/845,166, filed Apr. 21, 1997.]

US-CL-CURRENT: 424/757

ABSTRACT:

A method of reducing the percentage of Epstein-Barr virus genome-carrying cells which exhibit Epstein-Barr virus early antigen induction, where the Epstein-Barr virus genone-carrying cells have been cultivated in the presence of at least one tumor-promoting chemical. The method is carried out by cultivating Epstein-Barr virus genome-carrying cells in the presence of a tumor-promoting chemical; and treating the Epstein-Barr virus genome-carrying cells with betanins. The betanins are effectively reduce the incidence of Epstein-Barr virus early antigen induction in the cultivated Epstein-Barr virus genome-carrying cells.

4 Claims, 6 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 6

---------- KWIC ---------

Brief Summary Text - BSTX (15):

A first object of this invention is to evaluate a series of FDA-approved synthetic food colorants for inhibitory effects on carcinogenesis. The colorants were tested using in vitro assays for inhibition of Epstein-Barr

virus early antigen (EBV-EA) induction in cells exposed to the tumor promoter 12O-tetradecanoylphorbol13-acetate (TPA). High inhibitory activity toward EBV-EA induction is known to be indicative that a composition has potential utility as an anti-tumor promoting or cancer chemopreventive agent. Epstein-Barr virus genome-carrying lympholastoid cells (Raji cells) were used in the in vitro studies.

US-PAT-NO: 6203839

DOCUMENT-IDENTIFIER: US 6203839 B1
See image for Certificate of Correction

TITLE: Flavorant compositions

DATE-ISSUED: March 20, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Bachmann; Jean-Pierre	Wadenswil	N/A	N/A	CH
Gautschi; Markus	Zeiningen	N/A	N/A	CH
Hostettler; Bernhard	Gockhausen	N/A	N/A	CH
Yang; Xiaogen	West Chester	OH	N/A	N/A

APPL-NO: 09/ 212985

DATE FILED: December 16, 1998

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
EP	97122633	December 22, 1997

US-CL-CURRENT: 426/546, 426/538 , 426/638 , 426/650

ABSTRACT:

The invention is related to a flavorant composition containing 1'-acetoxychavicol acetate and 1'-acetoxyeugenol acetate derivatives thereof as well as a flavorant acceptable carrier. The flavorant composition may be used for flavoring foods, beverages or healthcare products with warm/hot, spicy and pungent sensations related to Galangal.

2 Claims, 0 Drawing figures

Exemplary Claim Number: 1

---------- KWIC ---------

Other Reference Publication - OREF (6):
Kondo et al., 1'Acetoxychavicol Acetate as a Potent Inhibitor of Tumor Promoter-induced Epstein-Barr Virus Activation from Languas galanga, a Traditional Thai Condiment, Biosci. Biotech. Biochem. 57(8) (1993) 1344-1345.

US-PAT-NO: 6200760

DOCUMENT-IDENTIFIER: US 6200760 B1

TITLE: Method of screening agents as candidates for drugs or sources of drugs

DATE-ISSUED: March 13, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Dannenberg; Andrew J.	New York	NY	N/A	N/A
Subbaramaiah; Kotha J.	Flushing	NY	N/A	N/A
Pasco; David S.	Oxford	MS	N/A	N/A

APPL-NO: 09/ 355940

DATE FILED: August 13, 1999

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is the national phase of PCT/US98100023, filed Jan. 12, 1998, which claims the benefit of U.S. Provisional Application No. 60/038,254, filed Feb. 24, 1997.

PCT-DATA:
APPL-NO: PCT/US98/00023
DATE-FILED: January 12, 1998
PUB-NO: WO98/37235
PUB-DATE: Aug 27, 1998
371-DATE: Aug 13, 1999
102(E)-DATE:Aug 13, 1999

US-CL-CURRENT: 435/6, 435/4 , 435/471 , 435/476 , 435/8 , 435/91.1

ABSTRACT:

Cells are transfected with a construct containing transcriptional promoter element(s) that have been implicated in carcinogenesis or inflammation ligated to a reporter gene. Determination of inhibition of activation of said promoter element(s) by putative agent indicates the agent is a candidate as a drug or source of a drug for prophylaxis or treatment of cancer or inflammation. The method has particular application to screening agents as candidates for drugs or sources of drugs for prophylaxis or treatment of human disorders caused or mediated by cyclooxygenase-2 and/or matrix metalloproteinases.

2 Claims, 12 Drawing figures

Exemplary Claim Number: 2

Number of Drawing Sheets: 6

---------- KWIC ---------

Detailed Description Text - DETX (23):

In a very preferred method herein for screening for candidates for treatment of an inflammatory and/or carcinogenic disorder, the cells that are transfected are chondrocytes and a battery of screenings are carried out on each putative compound, where in one case the chondrocytes are transfected with a construct containing the COX-2 promoter and the activator is phorbol myristate acetate used in combination with interleukin-1; in another case, the chondrocytes are transfected with Ets2/AP-1 transcription promoter elements and the activator used is phorbol myristate acetate; in another case, the chondrocytes are transfected with construct containing NF-.kappa.B transcription promoter element (a synthetic element containing the NF-.kappa.B motif from HIV/IgK) and the activator is interleukin-1; in another case, the chondrocytes are transfected with construct containing said NF-.kappa.B transcription promoter element and the activator is tumor necrosis factor-alpha; and in another case, the chondrocytes are transfected with construct containing said NF-.kappa.B transcription promoter element and the activator is phorbol myristate acetate. The more cases of inhibition of activation, the higher the likelihood of antiinflammatory and therefore anticancer property.

Detailed Description Text - DETX (42):

The following combinations of construct in transfected cells and inducer were utilized: (1) COX-2 promoter ligated to luciferase as construct and phorbol myristate acetate together with interleukin-1 as inducer; (2) Ets2/AP-1 ligated to luciferase as construct and phorbol myristate acetate as inducer; (3) NF-.kappa.B ligated to luciferase as construct and interleukin-1 as inducer; (4) NF-.kappa.B ligated to luciferase as construct and tumor necrosis factor-alpha as inducer; (5) NF-.kappa.B ligated to luciferase as construct and phorbol myristate acetate as inducer; and (6) Sp1-luciferase as construct and phorbol myristate acetate together with interleukin-1 inducer.

US-PAT-NO: 6103933

DOCUMENT-IDENTIFIER: US 6103933 A
See image for Certificate of Correction

TITLE: Methods for controlling the oxidation rate of a hydrocarbon by adjusting the ratio of the hydrocarbon to a rate-modulator

DATE-ISSUED: August 15, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
DeCoster; David C.	Buckley	WA	N/A	N/A
Rostami; Ader M.	Bainbridge Island	WA	N/A	N/A
Dassel; Mark W.	Indianola	WA	N/A	N/A
Vassiliou; Eustathios	Newark	DE	N/A	N/A

APPL-NO: 08/ 861180

DATE FILED: May 21, 1997

PARENT-CASE:

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims priority from U.S. Provisional Application No. 60/030,699 filed Nov. 7, 1996, which application is incorporated herein by reference in its entirety.

US-CL-CURRENT: 562/509

ABSTRACT:

Methods for controlling the oxidation rate of a hydrocarbon to an acid by adjusting addition of a rate-modulator are disclosed. In order to control oxidation rate, the ratio of hydrocarbon to rate modulator is appropriately adjusted. Preferably, this ratio is adjusted continually based on feedback relative to oxidation progress parameters. It may be kept substantially constant at steady state conditions of the oxidation, or it may take a path of predetermined values. The rate-modulator preferably comprises a hydrocarbon oxidation initiator.

91 Claims, 3 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 3

---------- KWIC ---------

Detailed Description Text - DETX (23):
In operation of this embodiment, hydrocarbon, cyclohexane for example, solvent, acetic acid for example, catalyst, cobalt acetate tetrahydrate for example, preferably in the form of a solution comprising solvent for example, and a rate modulator, such as an oxidation initiator or promoter, like

cyclohexanone or acetaldehyde, for example enter the major reaction chamber 12 as disclosed in our our co-pending applications, and/or patents. At the same time that the aforementioned ingredients enter the major reaction chamber 12, a gaseous oxidant also enters through line 18, and starts reacting with the hydrocarbon. No recyclables exist at the initial stages.

US-PAT-NO: 6074979

DOCUMENT-IDENTIFIER: US 6074979 A
See image for Certificate of Correction

TITLE: Polybetaine-stabilized, palladium-containing nanoparticles, a process for preparing them and also catalysts prepared from them for producing vinyl acetate

DATE-ISSUED: June 13, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Hagemeyer; Alfred	Frankfurt	N/A	N/A	DE
Dingerdissen; Uwe	Seeheim-Jugenheim	N/A	N/A	DE
Millauer; Hans	Eschborn	N/A	N/A	DE
Manz; Andreas	Sinzheim	N/A	N/A	DE
Kuhlein; Klaus	Kelkheim	N/A	N/A	DE

APPL-NO: 09/ 083008

DATE FILED: May 21, 1998

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
DE	197 21 601	May 23, 1997

US-CL-CURRENT: 502/159, 502/326 , 502/330 , 502/339

ABSTRACT:

Polybetaine-stabilized, palladium-containing nanoparticles, a process for preparing them and also catalysts prepared from them for producing vinyl acetate. The invention relates to soluble nanoparticles which comprise palladium alone or palladium together with metals of the groups 8-11 of the periodic table and which are embedded in protective colloids, wherein the protective colloids comprise at least one polymer having betaine groups, and to a process for preparing them. The soluble nanoparticles are suitable for the preparation of catalysts.

32 Claims, 0 Drawing figures

Exemplary Claim Number: 1

---------- KWIC ---------

Brief Summary Text - BSTX (74):

The carrier can also be loaded with further activators, especially alkali metal acetates, preferably potassium acetate and, where appropriate, promoters, for example Zr, Ti, Cd, Cu, Ba and Re compounds, before, during and/or after the immobilization of the sols.

US-PAT-NO: 6066488

DOCUMENT-IDENTIFIER: US 6066488 A

TITLE: Cathepsin C homolog

DATE-ISSUED: May 23, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Coleman; Roger	Mountain View	CA	N/A	N/A
Braxton; Scott Michael	San Mateo	CA	N/A	N/A
Seilhamer; Jeffrey J.	Los Altos Hills	CA	N/A	N/A

APPL-NO: 08/ 871314

DATE FILED: June 9, 1997

PARENT-CASE:

The present U.S. utility patent application is a divisional application of then U.S. Ser. No. 08/426,428, filed Apr. 19, 1995, now U.S. Pat. No. 5,637,462, from which priority is claimed.

US-CL-CURRENT: 435/212, 424/94.63 , 424/94.65 , 435/219 , 435/226 , 435/320.1 , 435/325 , 530/412 , 536/23.1 , 536/23.2

ABSTRACT:

The present invention provides nucleotide and amino acid sequences that identify and encode a new cathepsin C homolog (RCP) expressed in THP-1 cells. The present invention also provides for antisense molecules to the nucleotide sequences which encode RCP, expression vectors for the production of purified RCP, antibodies capable of binding specifically to RCP, hybridization probes or oligonucleotides for the detection of RCP-encoding nucleotide sequences, genetically engineered host cells for the expression of RCP, diagnostic tests for activation of monocyte/macrophages based on RCP-encoding nucleic acid molecules, and use of the protein to produce antibodies capable of binding specifically to the protein and use of the protein to screen for inhibitors.

4 Claims, 3 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 6

---------- KWIC ---------

Brief Summary Text - BSTX (8):

Many human myeloid and myelomonocytic cell lines retain some ability to differentiate into more mature phenotypes in response to various internal stimuli including growth factors, lymphokines, cytokines, vitamin D derivatives, and tumor promoters and external agents such as trauma, smoking, UV irradiation, asbestos exposure, and steroids. THP-1 cells treated with the tumor promoter 12-O-tetradecanoyl-phorbol-13 acetate (TPA) are induced to stop proliferating and differentiate into macrophage-like cells which mimic native

monocyte-derived macrophages both morphologically and physiologically.

US-PAT-NO: 5998188

DOCUMENT-IDENTIFIER: US 5998188 A

TITLE: Mitogen activated protein kinase phosphatase cDNAS and their biologically active expression products

DATE-ISSUED: December 7, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Stork; Philip J. S.	Portland	OR	N/A	N/A
Misra-Press; Anita	Portland	OR	N/A	N/A

APPL-NO: 08/ 990379

DATE FILED: December 15, 1997

PARENT-CASE:

RELATED APPLICATIONS

The present application claims priority under 35 U.S.C. .sctn.120 from PCT International Application No. PCT/US96/10402, filed Jun. 14, 1996, which is a continuation of U.S. Provisional Ser. No. 60/000,263, filed Jun. 16, 1995, all hereby expressly incorporated by reference.

US-CL-CURRENT: 435/196

ABSTRACT:

The invention relates to a novel mitogen-activated protein kinase phosphatase, MKP-2. The invention further relates to methods and means for preparing and to nucleic acids encoding this protein. The MKP-2 of the present invention is useful in the control of cell growth, differentiation and apoptosis.

3 Claims, 44 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 24

---------- KWIC ---------

Detailed Description Text - DETX (71):

ERK activity is reduced in MKP-1 and MKP-2 overexpressing cells. Growth factor, hormone, and phorbol ester stimulation of PC12 cells has been known to activate the MAP kinase pathway and to stimulate the enzymatic activity of ERK-1 (FIGS. 13A and 13B) (61, 62, 65, 69, 71). We compared the enzymatic activity of ERK-1, in wild type cells and in MKP overexpressing cells treated with these agents. PC12 cells treated for 10 minutes with mitogenic agents such as EGF, differentiating agents such as NGF and forskolin, and the tumor promoter phorbol 12-myristate 132-acetate (PMA), produce a robust activation of ERK-1 as measured by an immune complex activity assay (FIG. 13A). MKP-1 and MKP-2 overexpressing clones were then treated with the same agents for the

identical times. Both MKP1.10 and MKP2.3 cells lines showed a dramatic reduction in the ability of growth factors and hormones to activate ERK-1 (FIG. 13A). Additional positive clones were also analyzed and showed similar results (data not shown). Quantitation of the immune complex assays shows that the modest overexpression of MKP-1 and MKP-2 mRNA in PC12 cells inhibited growth factor and hormoneinduced activation of ERKs 80-90% in MKP2.3 cells and 50-80% in MKP1.0 cells compared to the fold activation seen.in wild type PC12 cells (FIG. 13B). The basal ERK activity also appeared to be lower in these MKP overexpression cells as compared to wild type cells (FIG. 13B), insert).

US-PAT-NO: 5972357

DOCUMENT-IDENTIFIER: US 5972357 A

TITLE: Healthy foods and cosmetics

DATE-ISSUED: October 26, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Yamaguchi; Fumio	Noda	N/A	N/A	JP
Saito; Makoto	Noda	N/A	N/A	JP
Ishikawa; Hiroharu	Noda	N/A	N/A	JP
Kataoka; Shigehiro	Noda	N/A	N/A	JP
Ariga; Toshiaki	Noda	N/A	N/A	JP

APPL-NO: 08/ 975713

DATE FILED: November 21, 1997

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
JP	8-353869	December 19, 1996
JP	9-199119	July 10, 1997
JP	9-199120	July 10, 1997

US-CL-CURRENT: 424/401, 514/675 , 514/678 , 514/690 , 514/724 , 514/729 , 514/730

ABSTRACT:

The present invention relates to healthy foods and cosmetics. More particularly, it relates to healthy foods and cosmetics containing a polyisoprenylated benzophenone derivatives as effective ingredients and having a variety of functions for maintaining health such as anti-ulcer activity, the Maillard reaction inhibiting activity, anti-oxidation activity, reactive oxygen species scavenging activity, and anti-tumor promotion activity.

5 Claims, 0 Drawing figures

Exemplary Claim Number: 1

---------- KWIC ---------

Detailed Description Text - DETX (53):

The EBV potentially infected human lymphoblasts (Raji) were first prepared in a concentration of 5.times.10.sup.5 cell/ml, and cultured in an RPMI-1640 medium to which 3 mM n-butyric acid (inducer) and 50 nM TPA (12-O-tetradecanoylphorbol-13-acetate) (promoter) were added in an atmosphere of 5% carbon dioxide and 95% air under at a temperature of 37.degree. C. for 48 hours. The cells in which EBV-EA had been induced were detected microscopically by indirect immunofluorescence with the serum of a nasopharyngeal carcinoma (NPC) patient. In this system, various concentrations (8 .mu.g/ml, 40 .mu.g/ml, and 200 .mu.g/ml) of the test substance (garcinol sample shown in Referential Example 1) dissolved in DMSO (dimethylsolfoxide) were added together with the promoter to the cells. The activity of

suppressing the induction of the Epstein-Barr virus early antigen was defined as the decreasing rate of the cells in which EBV-EA had been induced.

US-PAT-NO: 5955369

DOCUMENT-IDENTIFIER: US 5955369 A

TITLE: Method for the determination of mutant restriction enzymes

DATE-ISSUED: September 21, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Mallonee; Richard L.	Baltimore	MD	N/A	N/A

APPL-NO: 08/ 895074

DATE FILED: July 16, 1997

US-CL-CURRENT: 435/6, 435/18

ABSTRACT:

The present invention is directed to a method for the determination of mutant restriction enzymes which comprises incubating restriction enzymes under non-native conditions with a labeled double stranded oligonucleotide to a solid support to form an enzyme-oligonucleotide complex and detecting the label to determine cleavage of the oligonucleotide by mutant enzymes.

13 Claims, 1 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 1

---------- KWIC ---------

Detailed Description Text - DETX (38):

In this example, large numbers of bacterial colonies are screened for the production of mutant proteins/enzymes. A culture of bacteria carrying the mutated gene of interest is plated out as follows. A nitrocellulose membrane (NC) is prepared as in Example 1 by coating with the same BSA-biotin-streptavadin-biotin-oliognucleotide complex. The NC membrane is then placed on an agar plate containing LB medium (Luria-Bertani medium), antibiotic and isopropylthio-.beta.-D-galactoside (IPTG). A cellulose acetate filter is placed on top of the coated NC membrane. The bacteria, in this example, recombinant E. coli, (this strain harbors a vector containing the trc promoter for expression of HincII and is inducible with IPTG), is spread on the cellulose acetate filter at a dilution to form single colonies. These plates are then incubated at 37.degree. C. for at least 16 hours to allow bacterial colonies to form and grow. The cellulose acetate membrane containing the colonies is removed and retained for positive colony isolation. The NC membrane is removed and developed, using antibodies at dilutions described in Example 1. Membranes are washed five times with PBS. They are then developed with 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium (Sigma Fast BCIP/NBT made by Sigma Chemical Company) as a precipitable substrate. A "negative spot" (absence of color) created by a single colony on a background of precipitated color indicates the restriction enzyme being produced has

cleaved the double stranded oligonucleotide.

US-PAT-NO: 5910629

DOCUMENT-IDENTIFIER: US 5910629 A

TITLE: Chimeric genes comprising a fungus-responsive element

DATE-ISSUED: June 8, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Strittmatter; Gunter	Cologne	N/A	N/A	DE

APPL-NO: 08/ 737298

DATE FILED: January 6, 1997

PCT-DATA:
APPL-NO: PCT/EP95/00868
DATE-FILED: March 9, 1995
PUB-NO: WO96/28561
PUB-DATE: Sep 19, 1996
371-DATE: Jan 6, 1997
102(E)-DATE:Jan 6, 1997

US-CL-CURRENT: 800/279, 435/418 , 435/419 , 435/468 , 435/69.1 , 536/23.6 , 536/24.1 , 536/24.5 , 800/265 , 800/268 , 800/285 , 800/287 , 800/298

ABSTRACT:

The present invention provides improved fungus-responsive chimeric genes for the production of transgenic plants which have plant cells surrounding the site of fungal infection that become capable of killing, disabling or repelling the fungus or that are themselves killed or rendered unsuitable for the fungus to feed upon, thereby preventing the spread of the fungus infection.

27 Claims, 1 Drawing figures

Exemplary Claim Number: 1,8,11,20

Number of Drawing Sheets: 1

---------- KWIC ---------

Detailed Description Text - DETX (18):

Particularly preferred fungus-responsive prp1-1 elements of this invention are those termed delB34 (nucleotide positions 140 to 273 in SEQ ID No. 1), delB35 (nucleotide positions 100 to 273 in SEQ ID No. 1), and delB51 (nucleotide positions 176 to 273 in SEQ ID No. 1), that are significantly less responsive to salicylic acid and indolyl acetate application (2- to 3-fold lower induction) when compared to the 273 bp prp1-1 promoter, even more preferred fungus-responsive prp1-1 elements of this invention are those termed delX4 (nucleotide positions 1 to 239 in SEQ ID No. 1) and delX5 (nucleotide positions 1 to 153 in SEQ ID No. 1), since these portions have substantially (i.e., drastically) lost the expression observed in root tips with the 273 bp prp1-1 promoter (WO 93/119188, e.g., no histochemically detectable GUS protein

by a delX4- or delX5-GUS chimeric gene in roots), as well as being significantly less responsive to salicylic acid and indole acetate application (a 30- to 40-fold lower induction) when compared to the 273 bp prp1-1 promoter, while still retaining a significant fungal responsiveness. Also included in this definition are natural or artificial promoter elements with a DNA sequence that is substantially similar to any of the delX4, delX5, delB34, delB35, and delB51 DNA sequences defined above, i.e., having some nucleotides deleted, replaced or added provided substantially the same promoter characteristics are retained.

US-PAT-NO: 5874464

DOCUMENT-IDENTIFIER: US 5874464 A

TITLE: Conformationally constrained diacylglycerol analogues

DATE-ISSUED: February 23, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Marquez; Victor E.	Gaithersburg	MD	N/A	N/A
Lee; Jeewoo	Rockville	MD	N/A	N/A
Sharma; Rajiv	Rockville	MD	N/A	N/A
Wang; Shaomeng	Rockville	MD	N/A	N/A
Milne; George W. A.	Bethesda	MD	N/A	N/A
Nicklaus; Marc C.	Elkridge	MD	N/A	N/A
Blumberg; Peter M.	Frederick	MD	N/A	N/A
Lewin; Nancy E.	Rockville	MD	N/A	N/A

APPL-NO: 08/ 372602

DATE FILED: January 13, 1995

US-CL-CURRENT: 514/473, 514/372 , 514/424 , 514/439 , 514/471 , 548/214 , 548/550 , 548/551 , 549/321 , 549/323 , 549/40

ABSTRACT:

Conformationally constrained diacylglycerol analogues, pharmaceutical compositions comprising such analogues, and methods of using such analogues as agonists and antagonists of protein kinase C.

12 Claims, 0 Drawing figures

Exemplary Claim Number: 1

---------- KWIC ---------

Brief Summary Text - BSTX (7):

Tumor promoters, such as phorbol esters, e.g., 12-O-tetradecanoylphorbol-13-acetate (TPA), and aplysiatoxins, have been shown to activate PK-C by acting as stable, highly potent DAG equivalents. Unlike DAGS, whose presence in the cell membrane is transient, phorbol esters are not metabolized and are, therefore, able to activate PK-C chronically, bypassing the DAG pathway. Chronic constitutive activation of PK-C is associated with resistance of cancerous cells to antitumor drugs.

US-PAT-NO: 5851764

DOCUMENT-IDENTIFIER: US 5851764 A

TITLE: Human prostate tumor inducing gene-1 and uses thereof

DATE-ISSUED: December 22, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Fisher; Paul B.	Scarsdale	NY	N/A	N/A
Shen; Ruoqian	New York	NY	N/A	N/A

APPL-NO: 08/ 371377

DATE FILED: January 11, 1995

PARENT-CASE:

This application is a continuation-in-part of U.S application Ser. No. 08/351,888, filed Dec. 8, 1994, which is a continuation of U.S. Ser. No. 08/225,493, filed Apr. 11, 1994, now abandoned, which is a continuation of U.S. Ser. No. 08/106,323, filed Aug. 13, 1993, now abandoned, which is a continuation of U.S. Ser. No. 07/603,804, filed Oct. 25, 1990, now abandoned, the contents of which are hereby incorporated by reference.

US-CL-CURRENT: 435/6, 435/91.1 , 436/94 , 536/23.5 , 536/24.1 , 536/24.31 , 536/24.33 , 536/25.3

ABSTRACT:

This invention provides a method for preparing a hybridoma cell line which produces an antibody capable of specifically binding to a cell surface-expressed protein which expresses on the surface of one cell type but not the other. This invention also provides a method for preparing a hybridoma cell line which produces an antibody capable of specifically binding to a cell surface-expressed protein. This invention provides a method to prepare a hybridoma cell line which specifically recognizes and binds to a tumor associated antigen associated with a neoplastic, human cell. This invention also provides a method of preparing DNA encoding a cell surface antigen associated with a neoplastic, human cell. This invention further provides an isolated mammalian nucleic acid molecule having the sequence of Prostate Carcinoma Tumor Antigen Gene-1. This invention also provides an isolated mammalian nucleic acid molecules having the sequence of Prostate Tumor Inducing Gene-1. This invention provides an isolated mammalian nucleic acid molecules having the sequence of Prostate Tumor Inducing Gene-2. Finally, this invention provides an isolated mammalian nucleic acid molecules having the sequence of Prostate Tumor Inducing Gene-1.

8 Claims, 27 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 23

---------- KWIC ---------

Detailed Description Text - DETX (228):

PTI-1: Using primer sequences for bases present in the unique 630 bp 5' region of PTI-1 (A and L) and primer sequences corresponding to the elongation factor-1 alpha region of PTI-1 and RT-PCR approaches, the following additional information is currently available relative to PTI-1: (A) Tissue distribution studies (using tissue poly A.sup.+ mRNA blots from Clontech) have been performed using the A and L primers and a region corresponding to the elongation factor-1 alpha homologous region of PTI-1 as probes. The unique region of PTI-1 is only expressed in skeletal muscle and colon tissue, whereas the elongation factor-1 alpha hybridizes with an mRNA present in all of the tissue samples. These include, spleen, thymus, prostate, testis, ovary, small intestine, colon, peripheral blood leukocyte, heart, brain, placenta, lung, liver, skeletal muscle, kidney and pancreas. These studies reinforce our previous observations that the unique region of PTI-1 is not expressed in normal human prostate. (B) Expression of PTI-1 (A and L primers) is reduced in LNCaP cells treated with: a phorbol ester tumor promoter (12-0-tetradecanoyl-phorbol -13-acetate (TPA), that induces apoptosis in LNCaP cells; suramin; epidermal growth factor; transforming growth factor-alpha; or the synthetic androgen R1881. Using primers for prostate specific antigen (PSA) reductions in PTI-1 mRNA levels using the same agents are also apparent in LNCaP cells. These results suggest that similar changes inducing downregulation of PSA expression can also decrease PTI-1 expression in human prostate carcinoma cells. (C) Expression is apparent in human promyelocytic leukemia (HL-60) and an additional leukemic cell line K562. When induced to differentiate by TPA, PTI-1 expression decreases and is no longer apparent by 3 hr posttreatment in HL-60 cells. This change in mRNA levels after TPA treatment suggests that decreased expression of PTI-1 may be modulated as a function of growth arrest and terminal differentiation in HL-60 cells; (D) Expression is apparent in CREF cells transformed by diverse acting oncogenes, including wild-type 5 adenovirus (Ad5), mutant type 5 adenovirus (H5hr1), Ha-ras oncogene, v-src, human papilloma virus type 18 (HPV-18) and HPV-51. Using a dexamethasone (DEX) inducible Ad5 E1A transforming gene under the transcriptional control of a mouse mammary tumor virus promoter, expression of PTI-1 is only seen in the presence of DEX. Under these culturing conditions, DEX also results in EIA expression and transformation. These data indicate that induction of PTI-1 directly correlates with transformation induced by mechanistically different oncogenes. Figure of Northern blot; FIG. 11).

US-PAT-NO: 5837728

DOCUMENT-IDENTIFIER: US 5837728 A

TITLE: 9-cis retinoic acid esters and amides and uses thereof

DATE-ISSUED: November 17, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Purcell; William P.	Memphis	TN	N/A	N/A

APPL-NO: 08/ 380011

DATE FILED: January 27, 1995

US-CL-CURRENT: 514/529, 514/559 , 554/221

ABSTRACT:

Esters and amides of 9-cis-retinoic acid are synthesized, formulated into pharmaceutically acceptable carriers and administered for the treatment of acne vulgaris, cystic acne, hyper-pigmentation, hypo-pigmentation, psoriasis, dermal and epidermal hypoplasia and kerotoses, the reduction of wrinkling of the skin as an incident of aging and actinic damage, normalization of the production of sebum, the reduction of enlarged pores, promoting the rate of wound healing, limiting of scar tissue formation during healing and the like. They are additionally useful for treatment or amelioration of the same additional classes of skin disorders as is retinoic acid itself and other retinoids. These disorders include ichthyoses (e.g., ichthyosis hystrix, epidermolytic hyperkeratosis, and lamellar ichthyosis), follicular disorders (e.g., pseudofolliculites, senile comedones, nevus comidonicas, and trichostatis spinulosa), benign epithelial tumors (e.g., flat warts, trichoepithelioma, and molluscum contagiosum), perforated dematoses (e.g., elastosis perforans seripiginosa and Kyrles disease), and disorders of keratinization (e.g., Dariers disease, keratoderma, hyperkeratosis plantaris, pityriasis rubra pilaris, lichen planus acanthosis nigricans, and psoriasis). The esters and amides of 9-cis-retinoic acid are also effective for the non-irritating treatment of effects attributable to aging and particularly to photodamage and photoaging. The use of these compounds extends to non-irritating treatments involving the retardation and reversal of additional dermal and cosmetic conditions which are ameliorated by tretinoin such as the effacement of wrinkles, improvement in appearance, namely color and condition of the skin, spots caused from exposure to the sun as well as other skin disorders. The esters and amides of 9-cis-retinoic acid are exceptionally active when compared to other retinoids employed for such indications, and are also exceptionally safe in effective therapeutic doses in contrast to other retinoids.

40 Claims, 0 Drawing figures

Exemplary Claim Number: 1,12

---------- KWIC ---------

Detailed Description Text - DETX (63):
The ODC/Retinoid Bioassay is based on the method of Verma, A. K. and

Boutwell, R. K., Cancer Res. (1977) 37:2196-2201. The ODC assay measures a compounds effect on the prevention of the induction of ODC, namely the effect of the retinoid compound on the inhibition of the tumor promoter 12-O-tetradecanoylphorbol-13-acetate (TPA) induced ODC activity. The assay is carried out using CD-1 mice (aged 7 to 9 weeks). The dorsal hair of the mice is shaved 3-4 days before testing. Four mice are used for each point. The test retinoids, at one of two dose levels (1.7 and 17 nmoles) dissolved in 0.2 ml of acetone is applied topically to the back of each shaved mouse. A single dose of TPA (17 nM) is applied to the back of each treated mouse 30 minutes later. Control groups are treated with either acetone alone, TPA, or tretinoin. The mice are sacrificed by cervical dislocation 5 hours after TPA treatment.

US-PAT-NO: 5830692

DOCUMENT-IDENTIFIER: US 5830692 A

TITLE: Expression system which can be regulated

DATE-ISSUED: November 3, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Bock; August	Geltendorf	N/A	N/A	DE
Mayer; Dagmar	Munchen	N/A	N/A	DE
Schlensog; Verena	Ismaning	N/A	N/A	DE
Candussio; Anton	Munchen	N/A	N/A	DE

APPL-NO: 08/ 614686

DATE FILED: March 12, 1996

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
DE	195 10 930.9	March 24, 1995
DE	195 14 056.7	April 13, 1995

US-CL-CURRENT: 435/69.1, 435/252.3 , 435/252.33 , 435/320.1 , 536/23.1 , 536/24.1

ABSTRACT:

An expression system can be regulated by acetate, pH and oxygen, which expression system includes a trans-acting regulator protein and a promoter which can be activated by this protein. Any desired structural genes are maximally expressed under the control of the expression system at an oxygen partial pressure, pO.sub.2, of 0-5% and a pH of 6.0-6.5, and in the presence of acetate at a concentration of 40-60 mM. There is also a process for preparing this expression system, and a process for using this expression system.

12 Claims, 10 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 10

---------- KWIC ---------

Abstract Text - ABTX (1):

An expression system can be regulated by acetate, pH and oxygen, which expression system includes a trans-acting regulator protein and a promoter which can be activated by this protein. Any desired structural genes are maximally expressed under the control of the expression system at an oxygen partial pressure, pO.sub.2, of 0-5% and a pH of 6.0-6.5, and in the presence of acetate at a concentration of 40-60 mM. There is also a process for preparing this expression system, and a process for using this expression system.

Brief Summary Text - BSTX (17):

The present invention relates to an expression system which can be regulated by acetate, pH and oxygen, which expression system comprises a trans-acting regulator protein and a promoter which can be activated by this protein, wherein the regulator protein encompasses an amino acid sequence which is at least 75% homologous with the amino acid sequence SEQ ID NO:1, and the promoter encompasses a DNA sequence which is at least 95% homologous with the bases 315 to 397 of the DNA sequence SEQ ID NO:2.

Detailed Description Text - DETX (57):

In order to identify plasmids encoding a transacting factor which activates the bud promoter in the presence of acetate, E. coli BL142 was transformed with the Klebsiella terrigena gene library by means of electroporation (Fiedler and Wirth (1988) Analytical Biochemistry 170, pp. 38-44). The transformation mixtures were spread on so-called indicator plates (potassium phosphate-buffered TGYEP agar (pH 6.5) containing 0.4% glucose, 40 mM acetate, 1 mM X-Gal and ampicillin (100 .mu.g/ml)) and were incubated at 37.degree. C. Due to its very weak .beta.-galactosidase activity (Table 5), E. coli BL142 forms pale-blue colonies on these indicator plates. By contrast, after transformation, one clone formed a deep dark blue colony. It contained the plasmid pBAK1 (FIG. 4), which carries an approximately 1.8 kb-sized Sau3A fragment from Klebsiella terrigena. For the subsequent analyses, a 1.8 kb-sized HindIII fragment, which contains a 350 bp-sized fragment of vector pBR322 and a 1.45 kb-sized fragment from Klebsiella terrigena, was isolated from pBAK1 (See FIG. 4); the protruding ends of the fragment were filled in with Klenow polymerase. The fragment was then ligated, in both orientations, into plasmid pUC19 which had been linearized with SmaI. The resulting plasmids, which also impart a blue coloration to E. coli BL142 colonies on indicator plates, were designated pBAK14 and pBAK16 (FIGS. 5 and 6).

US-PAT-NO: 5808001

DOCUMENT-IDENTIFIER: US 5808001 A

TITLE: Human ice homolog antibodies and compositions thereof

DATE-ISSUED: September 15, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Braxton; Scott Michael	San Mateo	CA	N/A	N/A
Delegeane; Angelo M.	Hayward	CA	N/A	N/A
Diep; Dinh	San Francisco	CA	N/A	N/A

APPL-NO: 08/ 900491

DATE FILED: July 25, 1997

PARENT-CASE:

This application is a divisional application of application No. 08/443,865 filed May 31, 1995, now U.S. Pat. No. 5,654,146.

US-CL-CURRENT: 530/387.1, 530/388.1

ABSTRACT:

The present invention provides nucleotide and amino acid sequences that identify and encode a new human interleukin-1 converting enzyme homolog (ICEY). The present invention also provides for antisense molecules to the nucleotide sequences which encode ICEY, expression vectors for the production of purified ICEY, antibodies capable of binding specifically to ICEY, hybridization probes or oligonucleotides for the detection of ICEY-encoding nucleotide sequences, genetically engineered host cells for the expression of ICEY, diagnostic tests for activation of monocyte/macrophages based on ICEY-encoding nucleic acid molecules, and use of the protein to produce antibodies capable of binding specifically to the protein and use of the protein to screen for inhibitors.

2 Claims, 7 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 7

---------- KWIC ---------

Brief Summary Text - BSTX (21):

Many human myeloid and myelomonocytic cell lines retain some ability to differentiate into more mature phenotypes in response to various internal stimuli including growth factors, lymphokines, cytokines, vitamin D derivatives, and tumor promoters and external agents such as trauma, smoking, UV irradiation, asbestos exposure, and steroids. THP-1 cells treated with the tumor promoter 12-O-tetradecanoyl-phorbol-13 acetate (TPA) are induced to stop proliferating and differentiate into macrophage-like cells which mimic native monocyte-derived macrophages both morphologically and physiologically.

US-PAT-NO: 5654146

DOCUMENT-IDENTIFIER: US 5654146 A

TITLE: Human ice homolog

DATE-ISSUED: August 5, 1997

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Braxton; Scott Michael	San Mateo	CA	N/A	N/A
Delegeane; Angelo M.	Hayward	CA	N/A	N/A
Diep; Dinh	San Francisco	CA	N/A	N/A

APPL-NO: 08/ 443865

DATE FILED: May 31, 1995

US-CL-CURRENT: 435/6, 435/183 , 435/320.1 , 435/358 , 435/69.2 , 435/7.4 , 536/23.2

ABSTRACT:

The present invention provides nucleotide and amino acid sequences that identify and encode a new human interleukin-1 converting enzyme homolog (ICEY). The present invention also provides for expression vectors for the production of purified ICEY, hybridization probes for the detection of ICEY-encoding nucleotide sequences, genetically engineered host cells for the expression of ICEY, diagnostic tests for ICEY or for polynucleotides encoding ICEY, and use of the protein to produce antibodies capable of binding specifically to the protein and use of the protein to screen for inhibitors.

10 Claims, 6 Drawing figures

Exemplary Claim Number: 8

Number of Drawing Sheets: 7

---------- KWIC ---------

Brief Summary Text - BSTX (23):

Many human myeloid and myelomonocytic cell lines retain some ability to differentiate into more mature phenotypes in response to various internal stimuli including growth factors, lymphokines, cytokines, vitamin D derivatives, and tumor promoters and external agents such as trauma, smoking, UV irradiation, asbestos exposure, and steroids. THP-1 cells treated with the tumor promoter 12-O-tetradecanoyl-phorbol-13 acetate (TPA) are induced to stop proliferating and differentiate into macrophage-like cells which mimic native monocyte-derived macrophages both morphologically and physiologically.

US-PAT-NO: 5637462

DOCUMENT-IDENTIFIER: US 5637462 A

TITLE: Cathepsin C homolog

DATE-ISSUED: June 10, 1997

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Coleman; Roger	Mountain View	CA	N/A	N/A
Braxton; Scott M.	San Mateo	CA	N/A	N/A
Seilhamer; Jeffrey J.	Los Altos Hills	CA	N/A	N/A

APPL-NO: 08/ 426428

DATE FILED: April 19, 1995

US-CL-CURRENT: 435/6, 435/91.2 , 536/22.1 , 536/23.1 , 536/24.3 , 536/24.31

ABSTRACT:

The present invention provides nucleotide and amino acid sequences that identify and encode a new cathepsin C homolog (RCP) expressed in THP-1 cells. The present invention also provides for antisense molecules to the nucleotide sequences which encode RCP, expression vectors for the production of purified RCP, antibodies capable of binding specifically to RCP, hybridization probes or oligonucleotides for the detection of RCP-encoding nucleotide sequences, genetically engineered host cells for the expression of RCP, diagnostic tests for activation of monocyte/macrophages based on RCP-encoding nucleic acid molecules, and use of the protein to produce antibodies capable of binding specifically to the protein and use of the protein to screen for inhibitors.

5 Claims, 6 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 6

---------- KWIC ---------

Brief Summary Text - BSTX (8):

Many human myeloid and myelomonocytic cell lines retain some ability to differentiate into more mature phenotypes in response to various internal stimuli including growth factors, lymphokines, cytokines, vitamin D derivatives, and tumor promoters and external agents such as trauma, smoking, UV irradiation, asbestos exposure, and steroids. THP-1 cells treated with the tumor promoter 12-O-tetradecanoyl-phorbol-13 acetate (TPA) are induced to stop proliferating and differentiate into macrophage-like cells which mimic native monocyte-derived macrophages both morphologically and physiologically.

US-PAT-NO: 5591773

DOCUMENT-IDENTIFIER: US 5591773 A

TITLE: Inhibition of cataract formation, diseases resulting from oxidative stress, and HIV replication by caffeic acid esters

DATE-ISSUED: January 7, 1997

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Grunberger; Dezider	Teaneck	NJ	N/A	N/A
Frenkel; Krystyna	Woodmere	NY	N/A	N/A

APPL-NO: 08/ 212569

DATE FILED: March 14, 1994

US-CL-CURRENT: 514/532, 514/912

ABSTRACT:

A method of inhibiting the formation of a cataract in an eye by contacting the eye with a compound having the structure: ##STR1## is described. Also described is a method of inhibiting the progression of cataract formation in an eye. Methods comprising administering a pharmaceutical composition comprising the above compound to inhibit the formation of a cataract in the eye of a subject and to inhibit progression of cataract formation in the eye of a subject are also described. The above compound also prevents diseases resulting from oxidative stress, including diseases comprising tumor formation resulting from oxidative stress, and also inhibits the progression of diseases resulting from oxidative stress. The above compound may furthermore be used to treat an HIV infection when combined in a pharmaceutical composition with a substance which inhibits HIV replication.

30 Claims, 13 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 13

---------- KWIC ---------

Brief Summary Text - BSTX (5):

It has recently been shown that the phorbol ester-type tumor promoters (12-O-tetradecanoylphorbol-13-acetate, hereinafter TPA) induce H.sub.2 O.sub.2 production in mouse skin as well as cause oxidation of DNA bases in vivo (20-22). In addition, it has been found that agents possessing anti-tumor-promoting properties in vivo, also suppress inflammatory processes. Processes suppressed by such agents include infiltration of polymorphonuclear leukocytes (hereinafter PMNs), reactive oxygen species production, and oxidation of DNA bases (20-22), as well as induction of ornithine decarboxylase (ODC) and edema (23-26). A number of known anti-tumor promoters that possess all or some of those properties have been isolated from biological sources, and include sarcophytol A (isolate from marine soft coral) (27, 28),

(-)-epigallocatechin gallate (EGCG, a polyphenol from green tea) (26, 29, 30), curcumin (a spice) (24, 25), and caffeic acid (24, 25).

US-PAT-NO: 5374616

DOCUMENT-IDENTIFIER: US 5374616 A

TITLE: Compositions containing sphingosylphosphorylcholine and the use thereof as a cellular growth factor

DATE-ISSUED: December 20, 1994

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Spiegel; Sarah	Potomac	MD	N/A	N/A
Desai; Naishadh N.	Washington	DC	N/A	N/A

APPL-NO: 07/ 778662

DATE FILED: October 18, 1991

US-CL-CURRENT: 514/4, 435/405 , 514/114 , 514/12

ABSTRACT:

A pharmaceutical composition for promoting cellular proliferation in a mammal, which comprises:

a) an amount of an active ingredient effective to promote said cellular proliferation, and

b) a pharmaceutically acceptable carrier, said active ingredient comprising, at least, sphingosylphosphorylcholine.

3 Claims, 7 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 3

---------- KWIC ---------

Detailed Description Text - DETX (2):

In accordance with the present invention, it has been surprisingly discovered that sphingosylphosphorylcholine and pharmaceutical compositions containing the same exhibit a remarkably potent mitogenic effect for a wide variety of cell lines. Quite surprisingly, it has been discovered that sphingosylphosphorylcholine is a much more potent cellular growth factor than other known growth factors, including sphingosine and sphingosine-1-phosphate, and also acts synergistically with other agents such as insulin, epidermal growth factor (EGF), fibroblast growth factor (FGF) and the tumor promoter, 12-O-tetradecanoylphorbol-13-acetate (TPA), to induce cellular proliferation in mammalian cells.

US-PAT-NO: 5347046

DOCUMENT-IDENTIFIER: US 5347046 A

TITLE: Catalyst and process for using same for the preparation of unsaturated carboxylic acid esters

DATE-ISSUED: September 13, 1994

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
White; James F.	Hudson	OH	N/A	N/A
Slawski; Barbara	Parma	OH	N/A	N/A
White; Geoffrey	Shaker Heights	OH	N/A	N/A

APPL-NO: 08/ 067445

DATE FILED: May 25, 1993

US-CL-CURRENT: 560/245

ABSTRACT:

Disclosed are catalyst compositions comprising (i) one or more palladium group metals and/or compounds thereof; (ii) gold and/or compounds thereof; and (iii) optionally, an effective amount of one or more promoters selected from the group consisting of copper, nickel, cobalt, iron, manganese, lead, silver and compounds thereof. Preferably these catalyst compositions contain one or more promoters wherein the gram ratio of metal in the promoter to palladium group metal is up to about 0.4, wherein the catalysts are deposited on a support material and said support material is treated with one or more alkali metal bases and one or more alkali metal silicates prior to said deposition.

Also described are processes for preparing ethylenically unsaturated esters comprising reacting in the vapor phase at least one olefinic compound with at least one carboxylic organic acid and molecular oxygen in the presence of an effective amount of the foregoing catalyst compositions.

17 Claims, 0 Drawing figures

Exemplary Claim Number: 1

---------- KWIC ---------

Brief Summary Text - BSTX (35):

The catalysts of this invention may also contain one or more alkali or alkaline earth salts of an organic acid such as lithium, sodium, calcium or potassium acetate as a promoter and activator. These organic acid salts may be added to the catalyst in amounts up to 50 grams per liter of catalyst and preferably in the range of 15 to 35 grams per liter of catalyst.

US-PAT-NO: 5179056

DOCUMENT-IDENTIFIER: US 5179056 A

TITLE: Production of alkenyl alkanoate catalysts

DATE-ISSUED: January 12, 1993

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Bartley; William J.	Charleston	WV	N/A	N/A

APPL-NO: 07/ 793131

DATE FILED: November 18, 1991

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATION

This application is a continuation-in-part of U.S. patent application Ser. No. 696,215; filed May 6, 1991.

US-CL-CURRENT: 502/170, 502/330 , 560/241.1 , 560/245

ABSTRACT:

This invention provides a process for producing improved catalysts for the production of alkenyl alkanoates by the reaction of an alkene, an alkanoic acid and an oxygen-containing gas. The catalysts contain palladium, gold and a potassium promoter and are characterized by a reduced sodium content which results in increased catalyst activity. The reduced sodium content is obtained by using essentially sodium-free starting materials in the process for producing the catalysts.

6 Claims, 1 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 1

---------- KWIC ---------

Detailed Description Text - DETX (2):

In the practice of the process of the present invention, sodium-containing, water-soluble palladium and/or gold compounds can usually be employed since they are usually not used in amounts that result in catalysts having substantial amounts of sodium. The principal sources of sodium in alkenyl alkanoate catalysts are sodium-containing precipitating agents (e.g., sodium metasilicate) and/or sodium-containing promoters or activators (e.g., sodium acetate). To a lesser extent, some supports and some reducing agents (e.g., sodium borohydride) can introduce substantial amounts of sodium into the catalyst. Accordingly, in the practice of this invention, essentially sodium-free precipitating agents (e.g., potassium hydroxide), promoters (e.g., potassium acetate), reducing agents (e.g., hydrazine) and carriers are employed. When using potassium hydroxide as the precipitating agent, a

suitable potassium salt (e.g., potassium acetate) can also be used in the precipitating step to aid in displacement by potassium of any sodium bound on the carrier. Preferably the potassium hydroxide and the potassium salt are employed in an aqueous solution. The salt is used in an amount that provides from 1 to 10 weight percent potassium based on the total weight of the solution. Care should be exercised to ensure that the resulting catalyst does not contain so much potassium that catalyst activity is less than desired.

US-PAT-NO: RE34075

DOCUMENT-IDENTIFIER: US RE34075 E

TITLE: Dermal uses of trans-retinoids for the treatment of cancer

DATE-ISSUED: September 22, 1992

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Purcell; William P.	Memphis	TN	N/A	N/A
Parish; Harlie A.	Memphis	TN	N/A	N/A

APPL-NO: 07/ 732091

DATE FILED: July 18, 1991

REISSUE-DATA:

US-PAT-NO	DATE-ISSUED	APPL-NO	DATE-FILED
04994491	February 19, 1991	284185	December 14, 1988

PARENT-CASE:

.ladd.CROSS-REFERENCE TO RELATED APPLICATION

This application is a Continuation-in-Part of application Ser. No. 67,536, filed Jun. 29, 1987 (now U.S. Pat. No. 4,885,311, issued Dec. 5, 1989). .laddend.

US-CL-CURRENT: 514/529, 514/354 , 514/355 , 514/423 , 514/425 , 514/448 , 514/461 , 514/512 , 514/522 , 514/547 , 514/548 , 514/549

ABSTRACT:

The dermal use of non-irritating retinoids such as the esters and amides of 13-cis and 13-trans-retinoic acid for effecting the reduction and reversal of photoaging and skin cancer is disclosed.

5 Claims, 0 Drawing figures

Exemplary Claim Number: 1

---------- KWIC ---------

Detailed Description Text - DETX (5):

The ODC/Retinoid Bioassay is based on the method of Verma, A. K. and Boutwell, R. K., Cancer Res. (1977) 37:2196-2201. The ODC assay measures a compound's effect on the prevention of the induction of ODC, namely the effect of the retinoid compound on the inhibition of the tumor promoter 12-O-tetradecanoylphorbol-13-acetate (TPA) induced ODC activity. The assay was carried out using CD-1 mice (aged 7 to 9 weeks). The dorsal hair of the mice was shaved 3-4 days before testing. Four mice were used for each point. The test retinoids, at one of two dose levels (1.7 and 17 nmoles) dissolved in 0.2 ml of acetone was applied topically to the back of each shaved mouse. A single dose of TPA (17 nM) was applied to the back of each treated mouse 30 minutes

later. Control groups were treated with either acetone alone, TPA, or tretinoin. The mice were killed by cervical dislocation 5 hours after TPA treatment.

US-PAT-NO: 5124356

DOCUMENT-IDENTIFIER: US 5124356 A

TITLE: Dermal uses of trans-retinoids for the treatment of photoaging

DATE-ISSUED: June 23, 1992

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Purcell; William P.	Memphis	TN	N/A	N/A
Parish, Jr.; Harlie A.	Memphis	TN	N/A	N/A

APPL-NO: 07/ 609742

DATE FILED: November 6, 1990

PARENT-CASE:

CROSS REFERENCE TO A RELATED APPLICATION

This is a divisional of co-pending application Ser. No. 284,185, filed on Dec. 14, 1988, now U.S. Pat. No. 4,994,491, which is in turn a Continuation-in-Part of Applicants' co-pending application, Ser. No. 067,536, filed Jun. 29, 1987, now U.S. Pat. No. 4,885,311.

US-CL-CURRENT: 514/529, 514/354 , 514/355 , 514/423 , 514/425 , 514/448 , 514/461 , 514/512 , 514/522 , 514/547 , 514/548 , 514/549 , 514/725

ABSTRACT:

The dermal use of non-irritating retinoids such as the esters and amides of 13-cis and 13-trans-retinoic acid for effecting the reduction and reversal of photoaging and skin cancer is disclosed.

5 Claims, 0 Drawing figures

Exemplary Claim Number: 1

---------- KWIC ---------

Detailed Description Text - DETX (4):

The ODC/Retinoid Bioassay is based on the method of Verma, A. K. and Boutwell, R. K., Cancer Res. (1977) 37:2196-2201. The ODC assay measures a compound's effect on the prevention of the induction of ODC, namely the effect of the retinoid compound on the inhibition of the tumor promoter 12-0-tetradecanoylphorbol-13-acetate (TPA) induced ODC activity. The assay was carried out using CD-1 mice (aged 7 to 9 weeks). The dorsal hair of the mice was shaved 3-4 days before testing. Four mice were used for each point. The test retinoids, at one of two dose levels (1.7 and 17 nmoles) dissolved in 0.2 ml of acetone was applied topically to the back of each shaved mouse. A single dose of TPA (17 nM) was applied to the back of each treated mouse 30 minutes later. Control groups were treated with either acetone alone, TPA, or tretinoin. The mice were killed by cervical dislocation 5 hours after TPA

treatment.

US-PAT-NO: 5115096

DOCUMENT-IDENTIFIER: US 5115096 A
See image for Certificate of Correction

TITLE: Amphiregulin: a bifunctional growth modulating glycoprotein

DATE-ISSUED: May 19, 1992

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Shoyab; Mohammed	Seattle	WA	N/A	N/A
McDonald; Vicki L.	Kent	WA	N/A	N/A
Bradley; James G.	Woodinville	WA	N/A	N/A
Plowman; Gregory D.	Seattle	WA	N/A	N/A

APPL-NO: 07/ 297816

DATE FILED: January 17, 1989

PARENT-CASE:

The present application is a continuation-in-part of copending application Ser. No. 181,884 filed Apr. 15, 1988 which is a continuation-in-part of copending application Ser. No. 148,327 filed Jan. 25, 1988 both now abandoned each of which is incorporated by reference herein in its entirety.

US-CL-CURRENT: 530/322, 530/324

ABSTRACT:

A novel cell growth regulatory factor, named Amphiregulin, is described. This extremely hydrophilic glycoprotein, having a median molecular weight of 22,500 daltons, demonstrates unusual biological activity. Amphiregulin is a bifunctional cell growth regulatory factor which exhibits potent inhibitory activity on DNA synthesis in neoplastic cells, yet promotes the growth of certain normal cells. The invention is based, in part, on the discovery that MCF-7 cells, when treated with the tumor promoting agent, 12-O-tetradecanoyl-phorbol-13-acetate (TPA), express and secrete two distinct yet functionally equivalent forms of Amphiregulin. These two forms are structurally identical and perfectly homologous except that the truncated form lacks an amino-terminal hexapeptide found in the larger form. The Amphiregulin gene has been cloned and used to construct plasmids which direct the expression of bioactive Amphiregulin in transformed Escherichia coli cells. A wide variety of uses for Amphiregulin are encompassed by the present invention, including the treatment of wounds and cancers.

23 Claims, 21 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 35

---------- KWIC ---------

Brief Summary Text - BSTX (6):

Biologically active phorbol esters such as 12-0-tetradecanoyl-phorbol-13-acetate (TPA) are potent tumor-promoters in vivo and elicit and modulate a wide variety of biological and biochemical responses in vivo as well as in vitro (Blumberg, 1981, Crit. Rev. Toxicol. 9: 153-197; Slaga, 1983, Cancer Surv. 2: 595-612). It has been known for some time that TPA inhibits the growth of the human breast adenocarcinoma cell line MCF-7. In addition, TPA also alters the morphology of MCF-7 cells inasmuch as TPA treated cells exhibit the morphological characteristics of secretory cells (Osborne, et al., 1981, J. Clin. Invest. 67: 943-951; Valette et al., 1987, Cancer Res. 47: 1615-1620).

US-PAT-NO: 5049584

DOCUMENT-IDENTIFIER: US 5049584 A

TITLE: Dermal uses of cis-retinoids for the treatment of cancer

DATE-ISSUED: September 17, 1991

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Purcell; William P.	Memphis	TN	N/A	N/A
Parish, Jr.; Harlie A.	Memphis	TN	N/A	N/A

APPL-NO: 07/ 609609

DATE FILED: November 6, 1990

PARENT-CASE:

CROSS REFERENCE TO A RELATED APPLICATION

This is a divisional of co-pending application Ser. No. 284,185, filed on Dec. 14, 1988, now U.S. Pat. No. 4,994,491.

US-CL-CURRENT: 514/529, 514/354 , 514/355 , 514/423 , 514/425 , 514/448 , 514/461 , 514/512 , 514/522 , 514/547 , 514/548 , 514/549 , 514/725

ABSTRACT:

The dermal use of non-irritating retinoids such as the esters and amides of 13-cis and 13-trans-retinoic acid for effecting the reduction and reversal of photoaging and skin cancer is disclosed.

5 Claims, 0 Drawing figures

Exemplary Claim Number: 1

---------- KWIC ---------

Detailed Description Text - DETX (5):

The ODC/Retinoid Bioassay is based on the method of Verma, A. K. and Boutwell, R. K., Cancer Res. (1977) 37:2196-2201. The ODC assay measures a compound's effect on the prevention of the induction of ODC, namely the effect of the retinoid compound on the inhibition of the tumor promoter 12-O-tetradecanoylphorbol-13-acetate (TPA) induced ODC activity. The assay was carried out using CD-1 mice (aged 7 to 9 weeks). The dorsal hair of the mice was shaved 3-4 days before testing. Four mice were used for each point. The test retinoids, at one of two dose levels (1.7 and 17 nmoles) dissolved in 0.2 ml of acetone was applied topically to the back of each shaved mouse. A single dose of TPA (17 nM) was applied to the back of each treated mouse 30 minutes later. Control groups were treated with either acetone alone, TPA, or tretinoin. The mice were killed by cervical dislocation 5 hours after TPA treatment.

US-PAT-NO: 4994491

DOCUMENT-IDENTIFIER: US 4994491 A

TITLE: Dermal uses of trans-retinoids for the treatment of cancer

DATE-ISSUED: February 19, 1991

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Purcell; William P.	Memphis	TN	N/A	N/A
Parish, Jr.; Harlie A.	Memphis	TN	N/A	N/A

APPL-NO: 07/ 284185

DATE FILED: December 14, 1988

US-CL-CURRENT: 514/529, 514/354 , 514/355 , 514/423 , 514/425 , 514/448 , 514/461 , 514/512 , 514/522 , 514/547 , 514/548 , 514/549

ABSTRACT:

The dermal use of non-irritating retinoids such as the esters and amides of 13-cis and 13-trans-retinoic acid for effecting the reduction and reversal of photoaging and skin cancer is disclosed.

5 Claims, 0 Drawing figures

Exemplary Claim Number: 1

---------- KWIC ---------

Detailed Description Text - DETX (4):

The ODC/Retinoid Bioassay is based on the method of Verma, A. K. and Boutwell, R. K., Cancer Res. (1977) 37:2196-2201. The ODC assay measures a compound's effect on the prevention of the induction of ODC, namely the effect of the retinoid compound on the inhibition of the tumor promoter 12-O-tetradecanoylphorbol-13-acetate (TPA) induced ODC activity. The assay was carried out using CD-1 mice (aged 7 to 9 weeks). The dorsal hair of the mice was shaved 3-4 days before testing. Four mice were used for each point. The test retinoids, at one of two dose levels (1.7 and 17 nmoles) dissolved in 0.2 ml of acetone was applied topically to the back of each shaved mouse. A single dose of TPA (17 nM) was applied to the back of each treated mouse 30 minutes later. Control groups were treated with either acetone alone, TPA, or tretinoin. The mice were killed by cervical dislocation 5 hours after TPA treatment.

US-PAT-NO: 4923818

DOCUMENT-IDENTIFIER: US 4923818 A

TITLE: DNA clone of human type IV collagenase

DATE-ISSUED: May 8, 1990

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Goldberg; Gregory I.	St. Louis	MO	N/A	N/A
Eisen; Arthur Z.	St. Louis	MO	N/A	N/A

APPL-NO: 07/ 352069

DATE FILED: May 15, 1989

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATION

This is a continuation-in-part of copending application Ser. No. 07/93,421, filed Sept, 4, 1987.

US-CL-CURRENT: 435/226, 435/320.1 , 536/23.2 , 536/23.5

ABSTRACT:

The cDNA clone representing the full size human type IV collagenase (gelatinase) is disclosed.

5 Claims, 9 Drawing figures

Exemplary Claim Number: 5

Number of Drawing Sheets: 14

---------- KWIC ---------

Brief Summary Text - BSTX (12):

The gelatinase protein sequence consists of three domains, an amino terminal domain, I, of 192 amino acids, a middle domain, II, of 175 amino acids, and a carboxy terminal domain, III, of 264 amino acids. The outer domains I and II show homology to collagenase described in U.S. Pat. No. 4,772,557, the disclosure of which is incorporated herein by reference, and to stromelysin described by Wilhelm et al., Proc. Natl. Acad. Sci. USA, 84, 6725-6729 (1987). The middle domain II, 175 amino acids long, is organized into three 58 amino acid long head-to-tail repeats which show homology to the type II motif of the collagen binding domain of fibronectin. In contrast to the expression of human fibroblast collagenase and stromelysin, the expression of type IV collagenase (gelatinase) by a variety of human fibroblast cell strains is not modulated by the tumor promoter TPA (12-0-tetradecanolphorbol 13-acetate).

US-PAT-NO: 4708964

DOCUMENT-IDENTIFIER: US 4708964 A

TITLE: Lipoxygenase inhibitors

DATE-ISSUED: November 24, 1987

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Allen; Larry M.	Fort Collins	CO	N/A	N/A

APPL-NO: 06/ 578414

DATE FILED: February 9, 1984

US-CL-CURRENT: 514/533, 514/23 , 514/464 , 514/469 , 514/532 , 514/559 , 514/570 , 514/64 , 514/718 , 514/731 , 514/826 , 514/830 , 514/859 , 514/863

ABSTRACT:

This invention provides methods of using a number of compounds for the inhibition of lipoxygenase in humans. Pathological conditions which may be treated by the compounds described herein include psoriasis, cellular proliferation, skin allergies, insect bites, allergic rhinitis, conjunctivitis, hay fever, bronchial asthma, allergic gastroenteritis, uterine contractions, hyperactivity of the colon and bronchospasms.

6 Claims, 0 Drawing figures

Exemplary Claim Number: 1

---------- KWIC ---------

Other Reference Publication - OREF (24):
Satoshi Yamamoto, et al., "Tumor Promoter 12-O-Tetradecanoylphorbol-13-Acetate-Induced Insulin Secretion: Inhibition by Phospholipase A.sub.2 -And Lipoxygenase-Inhibitors", Biochemical and Biophysical Research Communications, vol. 105, No. 2, pp. 759-765, Mar. 30, 1982.

US-PAT-NO: 4169099

DOCUMENT-IDENTIFIER: US 4169099 A

TITLE: Catalyst and process for producing ethylene oxide

DATE-ISSUED: September 25, 1979

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Khoobiar; Sargis	Kinnelon	NJ	N/A	N/A

APPL-NO: 05/ 909327

DATE FILED: May 25, 1978

US-CL-CURRENT: 549/536, 502/348

ABSTRACT:

In the oxidation of ethylene with molecular oxygen, improved selectivity to the production of ethylene oxide is obtained by employing a silver catalyst containing greater than zero and up to about 300 ppm by weight of thallium, based on the total catalyst.

8 Claims, 1 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 1

---------- KWIC ---------

Detailed Description Text - DETX (18):

After the particles have been dried, it is an important part of the preparation process to activate the particles by heating to decompose the silver compound or complex and to reduce the resulting silver oxide in order to produce an active catalyst. The promoter metal compounds, such as thallium acetate, are also decomposed during the activation process. It is common practice to heat the particles gradually in the presence of air to temperatures in the range of 200.degree.-300.degree. C. or more and to retain that temperature until the activation is complete. After the catalysts have been activated, they may be used for the oxidation of ethylene to ethylene oxide.

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	6162	isoprenoid$1 or lycopene$1 or carotene$1 or astaxanthin$1 or phytoene$1 or isopentyl adj diphosphate or ipp	USPAT	OR	OFF	2004/02/16 15:03
L2	147	acetyl adj phosphate or acetylphosphate	USPAT	OR	OFF	2004/02/16 15:05
L3	219	acetyl adj phosphate or acetylphosphate	US-PGPUB; USPAT	OR	OFF	2004/02/16 15:05
L4	0	1 near4 (gene$1 or sequence$1) and (L2 or L3)	USPAT	OR	OFF	2004/02/16 15:07
L5	306498	acetate	USPAT	OR	OFF	2004/02/16 15:06
L6	122	1 near4 (gene$1 or sequence$1) and L5	US-PGPUB; USPAT	OR	OFF	2004/02/16 15:13
L7	0	1 near4 (gene$1 or sequence$1) and L2	US-PGPUB; USPAT	OR	OFF	2004/02/16 15:07
L8	1278906	induc$8 or regulat$8 or activat$8 or modulat$8	USPAT	OR	OFF	2004/02/16 15:13
L9	12	1 near4 (gene$1 or sequence$1) and (L5 same L8)	US-PGPUB; USPAT	OR	OFF	2004/02/16 15:13

US-PAT-NO: 6689601

DOCUMENT-IDENTIFIER: US 6689601 B2

TITLE: High growth methanotropic bacterial strain

DATE-ISSUED: February 10, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Koffas; Mattheos	Wilmington	DE	N/A	N/A
Odom; James M.	Kennett Square	PA	N/A	N/A
Schenzle; Andreas	Zurich	N/A	N/A	CH

APPL-NO: 09/ 934868

DATE FILED: August 22, 2001

PARENT-CASE:

This application claims the benefit of U.S. Provisional Application No. 60/229,858 filed Sep. 1, 2000.

US-CL-CURRENT: 435/247, 435/232 , 435/248 , 435/250 , 435/252.1 , 435/71.1 , 536/24.1

ABSTRACT:

A high growth methanotrophic bacterial strain capable of growth on a C1 carbon substrate has been isolated and characterized. The strain has the unique ability to utilize both methane and methanol as a sole carbon source and has been demonstrated to possess a functional Embden-Meyerhof carbon flux pathway. The possession of this pathway conveys an energetic advantage to the strain, making it particularly suitable as a production platform for the production of biomass from a C1 carbon source.

14 Claims, 6 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 6

---------- KWIC ---------

Brief Summary Text - BSTX (7):

The conversion of C1 compounds to complex molecules with C--C bonds is a difficult and capital intensive process by traditional chemical synthetic routes. Traditionally, methane is first converted to synthesis gas (mixtures of hydrogen, carbon monoxide and carbon dioxide), which is then used to produce other small molecular weight industrial precursors. Typically these are "commodity" type chemicals such as acetate, formaldehyde, or methanol. The basic problem is activation of the methane molecule which is thermodynamically very difficult to achieve by chemical means. "Activation" refers to the process of making the chemically unreactive methane molecule more reactive.

Brief Summary Text - BSTX (24):
In a more specific embodiment the present strain may comprise genes encoding isoprenoid synthesizing enzymes where the enzymes are selected from the group consisting of SEQ ID NO:62, 64, 66, 68, 70, 72, 74, 86, and 78.

Detailed Description Text - DETX (68):
Further down in the isoprenoid biosynthesis pathway, more genes are involved in the synthesis of specific isoprenoids. As an example, the crtN gene that was found in Heliobacillus mobilis (Proc. Natl. Acad. Sci. USA 95:14851-14856 (1998)) encodes a diapophytoene dehydrogenase that is a part of the carotenoid biosynthesis pathway.

Detailed Description Text - DETX (69):
Although some of the genes involved in isoprenoid pathways are well known, the presence of genes involved in the isoprenoid pathway of Methylomonas sp. is rare. It is surprising therefore to find all of the above mentioned genes in the present strain (SEQ ID NO:61-SEQ ID NO:78). Tgus suggests that the present strain will be useful for the production of a variety of terpenoids. Accordingly the invention provides a Methylomonas strain having the genes and gene products as set forth in SEQ ID NO:61-SEQ ID NO:78, encoding a D-1-deoxyxylulose-5-phosphate synthase, a D-1-deoxyxylulose-5-phosphate reductoisomerase, 2C-methyl-d-erythritol 2,4-cyclodiphosphate synthase, a 2C-methyl-d-erythritol cytidylyltransferase, a CTP synthase, a Geranyltranstransferase (also farnesyl-diphosphate synthase), a 4-diphosphocytidyl-2-C-methylerythritol kinase, and a diapophytoene dehydrogenase.

Detailed Description Text - DETX (91):
Carotenoid pigments play a role in terms of providing coloration for many aquatic fish and crustacean species as well as providing antioxidant benefit. (Nelis H. J., De Leenheer 1991. J. Appl. Bacteriol. 70:181-191). Methylomonas 16a, unlike many commercially utilized methanotrophs (i.e. Methylococcus capsulatus) has a natural carotenoid pigment production pathway which produces high levels of a pink pigment that is similar, but not structurally identical, with such high value carotenoids as astaxanthin. Modification of this pathway by addition of genes involved in the final steps of astaxanthin synthesis or other high value carotenoids will result in the ability of this strain to produce these carotenoids. In this way Methylomonas 16a will be uniquely useful as an animal feed production strain in which the ratios of protein/carbohydrate/pigments may be tailored to suit particular nutritional needs. In this way, Methylomonas may be utilized as a way to deliver higher value components to other sources of plant protein or carbohydrate and thus circumvent the problem of genetic engineering of these plants for the higher value traits.

Detailed Description Text - DETX (99):
In a similar fashion the genes encoding the key enzymes involved in isoprenoid or pigment synthesis may be modulated. For example, the present invention provides a number of genes encoding key enzymes in the terpenoid pathway leading to the production of pigments and smaller isoprenoid compounds. The isolated genes include the dxs and dsr genes, the ispA, D, E, F, and G genes, the pyrg gene, and crtN genes. In particular it may be useful to up-regulate the initial condensation of 3-carbon molecules (pyruvate and C1 aldehyde group, D-glyceraldehyde 3-Phosphate), to yield the 5-carbon compound (D-1-deoxyxylulose-5-phosphate) mediated by the dxs gene. Alternatively, if it

is desired to produce a specific non-pigmented isoprenoid, it may be desirable to disrupt various genes at the downstream end of the pathway. For example, it may be desirable to use gene disruption or antisense inhibition of the crtN gene (known to encode diapophytoene dehydrogenase) if a smaller, upstream terpenoid is the desired product of the pathway.

Detailed Description Text - DETX (122):

A number of genes encoding specific identifying enzymes were isolated and sequenced from Methylomonas 16a. These include distinguishing genes found in the Entner-Douderoff carbon flux pathway the Embden-Meyerhof carbon flux pathway, genes encoding a denitrification pathway, genes encoding an isoprenoid synthesis pathway, and genes encoding a pathway for the synthesis of exopolysaccharides. These genes were sequenced and functionally characterized by comparison of their respective sequences to information in public nucleic acid and protein databases according to the following procedures.

US-PAT-NO: 6566584

DOCUMENT-IDENTIFIER: US 6566584 B1

TITLE: Compositions and methods for altering an acetyl-CoA metabolic pathway of a plant

DATE-ISSUED: May 20, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Coughlan; Sean J.	Hockessin	DE	N/A	N/A

APPL-NO: 09/ 377307

DATE FILED: August 19, 1999

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application No. 60/097,255, filed Aug. 20, 1998.

US-CL-CURRENT: 800/281, 435/332 , 435/419 , 435/468 , 536/23.6 , 800/278

ABSTRACT:

Compositions and methods for altering the content of plant seeds are provided. The compositions comprise nucleotide sequences encoding the enzyme acetyl-CoA synthetase. Such compositions find use in increasing the biosynthesis of fatty acids and/or carotenoids in plants. By expressing the sequences utilizing seed-specific promoters, plant seed can be obtained having increased levels of oils, specialty oils, carotenoids, and amino acids.

8 Claims, 1 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 1

---------- KWIC ---------

Brief Summary Text - BSTX (15):

Compositions and methods for modulating a metabolic pathway of a cell, particularly those pathways utilizing acetyl-CoA as a starting material, are provided. Such metabolic pathways include fatty acid biosynthesis, synthesis of isoprenoid compounds, and production of amino acids. The compositions comprise nucleotide sequences encoding the enzyme acetyl-CoA synthetase (ACS). ACS catalyzes formation of acetyl-CoA. Acetyl-CoA is a precursor to fatty acids in plastids through a four-step process using the substrate acetate.

Detailed Description Text - DETX (10):

To further increase the flux into fatty acid biosynthesis, an antisense construct for a nucleotide sequence encoding a protein in the carotenoid

pathway can be utilized. Such nucleotide sequences include, IPP isomerase (Hahn et al. (1996) J. Bacteriol. 178:619-624 and the references cited therein, GenBank Accession Nos. U48963 and X82627, GenBank Accession No. U48962, GenBank Accession No. U4896 1, GenBank Accession No. X 14230); geranylgeranyl pyrophosphate synthase (Misawa et al. (1990) J. Bacteriol. 172:6704-6712 and Application WO 91/13078); phytoene synthase (Misawa et al. (1990) J. Bacteriol. 172:6704-6712, GenBank Accession No. D90087, Application WO 91/13078, Armstrong et al. (1989) Mol. Gen. Genet. 216:254-268, Armstrong, G. A. "Genetic Analysis and Regulation of Carotenoid Biosynthesis." In R. C. Blankenship, M. T. Madigan, and C. E. Bauer (ed.), Anoxygenic photosynthetic bacteria; advances in photosynthesis. Kluwer Academic Publishers, Dordrecht, The Netherlands, Armstrong et al. (1990) Proc. Natl. Acad. Sci. USA 87:9975-9979, Armstrong et al. (1993) Methods Enzymol. 214:297-311, Bartley and Scolnik (1993) J. Biol. Chem. 268:27518-27521, Bartley et al. (1992) J. Biol. chem. 267:5036-5039, Bramley et al. (1992) Plant J. 2:291-343, Ray et al.(1992) Plant Mol. Biol. 19:401-404, Ray et al. (1987) Nucleic Acids Res. 15:10587, Romer et al. (1994) Biochem. Biophys. Res. Commun. 196:1414-1421, Karvouni et al. (1995) Plant Molecular Biology 27:1153-1162, GenBank Accession Nos. U32636, Z37543, L37405, X95596, D58420, U32636, Z37543, X78814, X82458, S71770, L27652, L23424, X68017, L25812, M87280, M38424, X69172, X63873, and X60441, Armstrong, G. A. (1994) J. Bacteriol. 176:4795-4802 and the references cited therein); and, phytoene desaturase (Misawa et al. (1990) J. Bacteriol. 1 72:6704-6712, Application WO 91/13078, GenBank Accession Nos. L37405, X95596, D58420, X82458, S71770, and M87280). See, also, Misawa et al. (1990) J. of Bacteriology 172:6704-6712, E. P. 0393690 B1, U.S. Pat. No. 5,429,939, Bartley et al. (1992) J. Biol. Chem. 267:5036-5039, Bird et al. (1991) Biotechnology 9:635-639, and U.S. Pat. No. 5,304,478, which disclosures are herein incorporated by reference.

Detailed Description Text - DETX (13):

To stop the pathway, an antisense RNA can be utilized for the accumulation of a particular compound. Alternatively, homologous plant sequences or partial plant sequences can be used to stop the pathway. For example, for the production of lycopene, an antisense lycopene .epsilon.-cyclase and the coding sequence for lycopene .beta.-cyclase (Hugueney et al. (1995) Plant J. 8:417-424, Cunningham et al. (1994) Plant Cell 6:1107-1121, Scholnik and Bartley (1995) Plant Physiol. 108:1343) are utilized.

US-PAT-NO: 6534291

DOCUMENT-IDENTIFIER: US 6534291 B1
See image for Certificate of Correction

TITLE: Compositions and methods for fumonisin detoxification

DATE-ISSUED: March 18, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Duvick; Jon	Des Moines	IA	N/A	N/A
Maddox; Joyce	Des Moines	IA	N/A	N/A
Gilliam; Jacob	Norwalk	IA	N/A	N/A
Folkerts; Otto	Guilford	CT	N/A	N/A
Crasta; Oswald R.	Branford	CT	N/A	N/A

APPL-NO: 09/ 677682

DATE FILED: October 2, 2000

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS

This Application is a Divisional Application of U.S. patent application Ser. No. 09/351,224, filed Jul. 12, 1999 issued as U.S. Pat. No. 6,388,171, herein incorporated by reference.

US-CL-CURRENT: 435/71.1, 424/93.1 , 435/196 , 435/252.3 , 435/320.1 , 536/23.1 , 536/23.2 , 536/24.1

ABSTRACT:

Compositions and methods for the complete detoxification of fumonisin and fumonisin degradation products are provided. Particularly, nucleotide sequences corresponding to the detoxification enzymes are provided. The sequences find use in preparing expression cassettes for the transformation of a broad variety of host cells and organisms.

21 Claims, 2 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 2

---------- KWIC ---------

Detailed Description Text - DETX (110):
Liquid cultures of Exophiala spinifera isolate 2141.10 were prepared from YPD agar plates (Yeast Extract 10 gm, Bacto-Peptone 20 gm, Dextrose 0.5 gm, Bacto-Agar 15 gm per liter of water). Aliquots (400-500 uL) of a water suspension of E. spinifera cells from YPD agar were spread uniformly onto 150.times.15 mm YPD agar plates with 4 mm sterile glass beads. The plates were incubated at room temperature for 6-7 days. The mycelia/conidia were transferred from the agar plates into Mineral Salts Medium (MSM) (Na.sub.2

HPO.sub.4.7H.sub.2 O 0.2 gm, NH.sub.4 Cl 1.0 gm, CaCl.sub.2.2H.sub.2 O 0.01 gm, FeSO.sub.4.7H.sub.2 O 0.02 gm per liter of distilled water, pH 4.5) and centrifuged at 5000.times.g, 4.degree. C., 20 minutes to pellet the cells. The cell pellet was rinsed once in 40 mL MSM and recentrifuged. The rinsed cell pellet was used to inoculate MSM at a 1:19 ratio of packed cells:MSM. The culture was supplemented with AP1 to a final concentration of 0.5-1.0 mg/ml and incubated at 28.degree. C., 100 rpm, in the dark to induce catabolic enzymes. The supernatants were removed by filtration through 0.45 cellulose acetate. The remaining mycelial mat was washed with sterile MSM and then frozen in liquid nitrogen for storage.

Other Reference Publication - OREF (61):
Schmidt et al. (1990) "Cloning and Nucleotide Sequence Of Thecrtl Gene Encoding Phytoene Dehydrogenase From The CyanobacteriumAphanocapsa PCC6714", Gene 91(1):113-117.

US-PAT-NO: 6482621

DOCUMENT-IDENTIFIER: US 6482621 B1

TITLE: Compositions and methods for fumonisin detoxification

DATE-ISSUED: November 19, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Duvick; Jon	Des Moines	IA	N/A	N/A
Maddox; Joyce	Des Moines	IA	N/A	N/A
Gilliam; Jacob	Norwalk	IA	N/A	N/A
Folkerts; Otto	Guilford	CT	N/A	N/A
Crasta; Oswald R.	Branford	CT	N/A	N/A

APPL-NO: 09/ 677488

DATE FILED: October 2, 2000

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS

This Application is a Divisional Application of U.S. patent application Ser. No. 09/351,224, filed Jul. 12, 1999 now issued as U.S. Pat. No. 6,388,171, herein incorporated by reference.

US-CL-CURRENT: 435/189, 435/440 , 536/23.2

ABSTRACT:

Compositions and methods for the complete detoxification of fumonisin and fumonisin degradation products are provided. Particularly, nucleotide sequences corresponding to the detoxification enzymes are provided. The sequences find use in preparing expression cassettes for the transformation of a broad variety of host cells and organisms.

6 Claims, 2 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 2

---------- KWIC ---------

Detailed Description Text - DETX (110):

Liquid cultures of Exophiala spinifera isolate 2141.10 were prepared from YPD agar plates (Yeast Extract 10 gm, Bacto-Peptone 20 gm, Dextrose 0.5 gm, Bacto-Agar 15 gm per liter of water). Aliquots (400-500 uL) of a water suspension of E. spinifera cells from YPD agar were spread uniformly onto 150.times.15 mm YPD agar plates with 4 mm sterile glass beads. The plates were incubated at room temperature for 6-7 days. The mycelia/conidia were transferred from the agar plates into Mineral Salts Medium (MSM) (Na.sub.2 HPO.sub.4 7H.sub.2 O 0.2 gm, NH.sub.4 Cl 1.0 gm, CaCl.sub.2 2H.sub.2 O 0.01 gm, FeSO.sub.4 7H.sub.2 O 0.02 gm per liter of distilled water, pH 4.5) and

centrifuged at 5000.times.g, 4.degree. C., 20 minutes to pellet the cells. The cell pellet was rinsed once in 40 mL MSM and recentrifuged. The rinsed cell pellet was used to inoculate MSM at a 1:19 ratio of packed cells: MSM. The culture was supplemented with AP1 to a final concentration of 0.5-1.0 mg/ml and incubated at 28.degree. C., 100 rpm, in the dark to induce catabolic enzymes. The supernatants were removed by filtration through 0.45 cellulose acetate. The remaining mycelial mat was washed with sterile MSM and then frozen in liquid nitrogen for storage.

Other Reference Publication - OREF (60):
Schmidt et al. (1990) "Cloning and Nucleotide Sequence Of Thecrti Gene Encoding Phytoene Dehydrogenase From The CyanobacteriumAphanocapsa PCC6714",Gene 91(1):113-117.

US-PAT-NO: 6455281

DOCUMENT-IDENTIFIER: US 6455281 B1

TITLE: Nucleic acids for identifying anti-fungal agents, and uses related thereto

DATE-ISSUED: September 24, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Berlin; Vivian	Dunstable	MA	N/A	N/A
Damagnez; Veronique	Cambridge	MA	N/A	N/A
Smith; Susan E.	Boston	MA	N/A	N/A

APPL-NO: 08/ 771212

DATE FILED: December 20, 1996

PARENT-CASE:

This application is a continuation-in-part of U.S. Application Ser. No. 08/631,319, filed Apr. 11, 1996, now U.S. Pat. No. 6,117,641.

US-CL-CURRENT: 435/69.2, 435/252.3 , 435/254.11 , 435/320.1 , 435/325 , 435/69.1 , 536/23.1 , 536/23.2 , 536/23.74

ABSTRACT:

The present invention relates to rapid, reliable and effective assays for screening and identifying pharmaceutically effective compounds that specifically inhibit the biological activity of fungal GTPase proteins, particularly GTPases involved in cell wall integrity, hyphael formation, and/or other cellular functions critical to pathogenesis. Another aspect of the present invention relates to novel Candida genes and gene products.

29 Claims, 21 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 14

---------- KWIC ---------

Detailed Description Text - DETX (42):

Still a variety of other formats exist which are amenable to high through put analysis on microtitre plates or the like. The prenylation substrate can be immobilized throughout the reaction, such as by cross-linking to activated polymer, or sequestered to the well walls after the development of the prenylation reaction. In one illustrative embodiment, a Rho-like GTPase, e.g. a fungal Rho1, Rho2, Cdc42 or Rsr1/Bud1, is cross-linked to the polymeric support of the well, the prenylation system set up in that well, and after completion, the well washed and the amount of geranylgeranyl sidechains attached to the immobilized GTPase detected. In another illustrative embodiment, wells of a microtitre plate are coated with streptavidin and contacted with the developed prenylation system under conditions wherein a

biotinylated substrate binds to and is sequestered in the wells. Unbound material is washed from the wells, and the level of prenylated target substrate is detected in each well. There are, as evidenced by this specification, a variety of techniques for detecting the level of prenylation of the immobilized substrate. For example, by the use of dansylated (described infra) or radiolabelled geranylgeranyl diphosphaste in the reaction mixture, addition of appropriate scintillant to the wells will permit detection of the label directly in the microtitre wells. Alternatively, the substrate can be released and detected, for example, by any of those means described above, e.g. by radiolabel, gel electrophoresis, etc. Reversibly bound substrate, such as the biotin-conjugated substrate set out above, is particularly amenable to the latter approach. In other embodiments, only the geranylgeranyl moiety is released for detection. For instance, the thioether linkage of the isoprenoid with the substrate peptide sequence can be cleaved by treatment with methyl iodide. The released geranylgeranyl products can be detected, e.g., by radioactivity, HPLC, or other convenient format.

Detailed Description Text - DETX (163):

The abbreviations used in Example 1 are: PKC, protein kinase C; MAPK, mitogen-activated protein kinase; MEK, MAPK-activating kinase; MEKK, MEK-activating kinase; DAG, diacylglycerol; SRF, serum response factor; JNK, Jun NH.sub.2 -terminal kinase (also known as SAPK, stress-activated protein kinase); PCR, polymerase chain reaction; HA, influenza hemagglutinin; PAGE, polyacrylamide gel electrophoresis; GST, glutathione-S-transferase; PS, phosphatidylserine; PMA, phorbol myristate acetate; GS, 1,3-.beta.-glucan synthase; MBP, myelin basic protein.

US-PAT-NO: 6388171

DOCUMENT-IDENTIFIER: US 6388171 B1
See image for Certificate of Correction

TITLE: Compositions and methods for fumonisin detoxification

DATE-ISSUED: May 14, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Duvick; Jon	Des Moines	IA	N/A	N/A
Maddox; Joyce	Des Moines	IA	N/A	N/A
Gilliam; Jacob	Norwalk	IA	N/A	N/A
Folkerts; Otto	Guilford	CT	N/A	N/A
Crasta; Oswald R.	Branford	CT	N/A	N/A

APPL-NO: 09/ 351224

DATE FILED: July 12, 1999

US-CL-CURRENT: 800/279, 435/320.1 , 435/418 , 435/419 , 435/468 , 435/69.1 , 536/23.2 , 536/23.7 , 536/24.1 , 800/278 , 800/287 , 800/288 , 800/306 , 800/312 , 800/314 , 800/317.4 , 800/320 , 800/320.1 , 800/320.2 , 800/320.3 , 800/322

ABSTRACT:

Compositions and methods for the complete detoxification of fumonisin and fumonisin degradation products are provided. Particularly, nucleotide sequences corresponding to the detoxification enzymes are provided. The sequences find use in preparing expression cassettes for the transformation of a broad variety of host cells and organisms.

46 Claims, 2 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 2

---------- KWIC ---------

Detailed Description Text - DETX (108):

Liquid cultures of Exophiala spinifera isolate 2141.10 were prepared from YPD agar plates (Yeast Extract 10 gm, Bacto-Peptone 20 gm, Dextrose 0.5 gm, Bacto-Agar 15 gm per liter of water). Aliquots (400-500 uL) of a water suspension of E. spinifera cells from YPD agar were spread uniformly onto 150.times.15 mm YPD agar plates with 4 mm sterile glass beads. The plates were incubated at room temperature for 6-7 days. The mycelia/conidia were transferred from the agar plates into Mineral Salts Medium (MSM) (Na.sub.2 HPO.sub.4.7H.sub.2 O 0.2 gm, NH.sub.4 Cl 1.0 gm, CaCl.sub.2.2H.sub.2 O 0.01 gm, FeSO.sub.4.7H.sub.2 O 0.02 gm per liter of distilled water, pH 4.5) and centrifuged at 5000.times.g, 4.degree. C., 20 minutes to pellet the cells. The cell pellet was rinsed once in 40 mL MSM and recentrifuged. The rinsed cell pellet was used to inoculate MSM at a 1:19 ratio of packed cells: MSM. The culture was supplemented with AP1 to a final concentration of 0.5-1.0 mg/ml

and incubated at 28.degree. C., 100 rpm, in the dark to induce catabolic enzymes. The supernatants were removed by filtration through 0.45 cellulose acetate. The remaining mycelial mat was washed with sterile MSM and then frozen in liquid nitrogen for storage.

Other Reference Publication - OREF (57):
Schmidt et al. (1990) "Cloning and Nucleotide Sequence Of Thecrtl Gene Encoding Phytoene Dehydrogenase From The Cyanobacterium Aphanocapsa PCC6714", Gene 91(1):113-117.

US-PAT-NO: 6277564

DOCUMENT-IDENTIFIER: US 6277564 B1

TITLE: Assays and reagents for identifying anti-fungal agents, and uses related thereto

DATE-ISSUED: August 21, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Berlin; Vivian	Dunstable	MA	N/A	N/A
Damagnez; Veronique	Cambridge	MA	N/A	N/A
Smith; Susan E.	Boston	MA	N/A	N/A

APPL-NO: 08/ 838973

DATE FILED: April 23, 1997

PARENT-CASE:

RELATED APPLICATIONS

This application is a continuation-in-part of Ser. No. 08/771,212 filed Dec. 20, 1996, which is a continuation-in-part of Ser. No. 08/631,319 filed Apr. 11, 1996 now U.S. Pat. No. 6,117,641, the specification of each of which is incorporated by reference herewith.

US-CL-CURRENT: 435/6, 536/22.1

ABSTRACT:

The present invention relates to rapid, reliable and effective assays for screening and identifying pharmaceutically effective compounds that specifically inhibit the biological activity of fungal GTPase proteins, particularly GTPases involved in cell wall integrity, hyphael formation, and/or other cellular functions critical to pathogenesis. Another aspect of the present invention relates to novel Candida genes and gene products.

5 Claims, 24 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 18

---------- KWIC ---------

Detailed Description Text - DETX (42):

Still a variety of other formats exist which are amenable to high through put analysis on microtitre plates or the like. The prenylation substrate can be immobilized throughout the reaction, such as by cross-linking to activated polymer, or sequestered to the well walls after the development of the prenylation reaction. In one illustrative embodiment, a Rho-like GTPase, e.g. a fungal Rho1, Rho2, Cdc42 or Rsr1/Bud1. is cross-linked to the polymeric support of the well, the prenylation system set up in that well, and after completion, the well washed and the amount of geranylgeranyl sidechains

attached to the immobilized GTPase detected. In another illustrative embodiment, wells of a microtitre plate are coated with streptavidin and contacted with the developed prenylation system under conditions wherein a biotinylated substrate binds to and is sequestered in the wells. Unbound material is washed from the wells, and the level of prenylated target substrate is detected in each well. There are, as evidenced by this specification, a variety of techniques for detecting the level of prenylation of the immobilized substrate. For example, by the use of dansylated (described infra) or radiolabelled geranylgeranyl diphosphaste in the reaction mixture, addition of appropriate scintillant to the wells will permit detection of the label directly in the microtitre wells. Alternatively, the substrate can be released and detected, for example, by any of those means described above, e.g. by radiolabel, gel electrophoresis, etc. Reversibly bound substrate, such as the biotin-conjugated substrate set out above, is particularly amenable to the latter approach. In other embodiments, only the geranylgeranyl moiety is released for detection. For instance, the thioether linkage of the isoprenoid with the substrate peptide sequence can be cleaved by treatment with methyl iodide. The released geranylgeranyl products can be detected, e.g., by radioactivity, HPLC, or other convenient format.

Detailed Description Text - DETX (162):

The abbreviations used in Example 1 are: PKC, protein kinase C; MAPK, mitogen-activated protein kinase; MEK, MAPK-activating kinase; MEKK, MEK-activating kinase; DAG, diacylglycerol; SRF, serum response factor; JNK, Jun NH.sub.2 -terminal kinase (also known as SAPK, stress-activated protein kinase); PCR, polymerase chain reaction; HA, influenza hemagglutinin; PAGE, polyacrylamide gel electrophoresis; GSt, glutathione-S-transferase; PS, phosphatidylserine; PMA, phorbol myristate acetate; GS, 1,3-.beta.-glucan synthase; MBP, myelin basic protein.

US-PAT-NO: 6271197

DOCUMENT-IDENTIFIER: US 6271197 B1

TITLE: Assays and reagents for identifying anti-fungal agents, and uses related thereto

DATE-ISSUED: August 7, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Berlin; Vivian	Dunstable	MA	N/A	N/A
Levin; David E.	Owens Mills	MD	N/A	N/A
Ohya; Yoshikazu	Tokyo	N/A	N/A	JP
Damagnez; Veronique	Cambridge	MA	N/A	N/A
Smith; Susan E.	Boston	MA	N/A	N/A

APPL-NO: 08/ 842306

DATE FILED: April 23, 1997

PARENT-CASE:

RELATED APPLICATIONS

This application is a continuation-in-part of 08/771,212 filed Dec. 20, 1996, which is a continuation-in-part of 08/631,319 filed Apr. 11, 1996, now U.S. Pat. No. 6,117,641, the specification of each of which is incorporated by reference herewith.

US-CL-CURRENT: 514/2, 435/15 , 435/7.1 , 435/7.4

ABSTRACT:

The present invention relates to rapid, reliable and effective assays for screening and identifying pharmaceutically effective compounds that specifically inhibit the biological activity of fungal GTPase proteins, particularly GTPases involved in cell wall integrity, hyphael formation, and/or other cellular functions critical to pathogenesis. Another aspect of the present invention relates to novel Candida genes and gene products.

40 Claims, 24 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 18

---------- KWIC ---------

Detailed Description Text - DETX (42):

Still a variety of other formats exist which are amenable to high through put analysis on microtitre plates or the like. The prenylation substrate can be immobilized throughout the reaction, such as by cross-linking to activated polymer, or sequestered to the well walls after the development of the prenylation reaction. In one illustrative embodiment, a Rho-like GTPase, e.g. a fungal Rho1, Rho2, Cdc42 or Rsr1/Bud1, is cross-linked to the polymeric

support of the well, the prenylation system set up in that well, and after completion, the well washed and the amount of geranylgeranyl sidechains attached to the immobilized GTPase detected. In another illustrative embodiment, wells of a microtitre plate are coated with streptavidin and contacted with the developed prenylation system under conditions wherein a biotinylated substrate binds to and is sequestered in the wells. Unbound material is washed from the wells, and the level of prenylated target substrate is detected in each well. There are, as evidenced by this specification, a variety of techniques for detecting the level of prenylation of the immobilized substrate. For example, by the use of dansylated (described infra) or radiolabelled geranylgeranyl diphosphaste in the reaction mixture, addition of appropriate scintillant to the wells will permit detection of the label directly in the microtitre wells. Alternatively, the substrate can be released and detected, for example, by any of those means described above, e.g. by radiolabel, gel electrophoresis, etc. Reversibly bound substrate, such as the biotin-conjugated substrate set out above, is particularly amenable to the latter approach. In other embodiments, only the geranylgeranyl moiety is released for detection. For instance, the thioether linkage of the isoprenoid with the substrate peptide sequence can be cleaved by treatment with methyl iodide. The released geranylgeranyl products can be detected, e.g., by radioactivity, HPLC, or other convenient format.

Detailed Description Text - DETX (162):

The abbreviations used in Example 1 are: PKC, protein kinase C MAPK, mitogen-activated protein kinase; MEK, MAPK-activating kinase MEKK, MEK-activating kinase; DAG, diacylglycerol; SRF, serum response factor; JNK, Jun NH.sub.2 -terminal kinase (also known as SAPK, stress-activated protein kinase); PCR, polymerase chain reaction, HA, influenza hemagglutinin; PAGE, polyacrylamide gel electrophoresis; GST glutathione-S-transferase; PS, phosphatidylserine; PMA, phorbol myristate acetate; GS, 1,3-.beta.-glucan synthase; MBP, myelin basic protein.

US-PAT-NO: 6235514

DOCUMENT-IDENTIFIER: US 6235514 B1

TITLE: Nucleic acid molecules encoding isopentenyl monophosphate kinase, and methods of use

DATE-ISSUED: May 22, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Croteau; Rodney B.	Pullman	WA	N/A	N/A
Lange; Bernd M.	Pullman	WA	N/A	N/A

APPL-NO: 09/ 434774

DATE FILED: November 4, 1999

US-CL-CURRENT: 435/194, 435/183 , 435/252.3 , 435/320.1 , 435/419 , 514/44 , 536/23.6

ABSTRACT:

A cDNA encoding isopentenyl monophosphate kinase (IPK) from peppermint (Mentha x piperita) has been isolated and sequenced, and the corresponding amino acid sequence has been determined. Accordingly, an isolated DNA sequence (SEQ ID NO:1) is provided which codes for the expression of isopentenyl monophosphate kinase (SEQ ID NO:2), from peppermint (Mentha x piperita). In other aspects, replicable recombinant cloning vehicles are provided which code for isopentenyl monophosphate kinase, or for a base sequence sufficiently complementary to at least a portion of isopentenyl monophosphate kinase DNA or RNA to enable hybridization therewith. In yet other aspects, modified host cells are provided that have been transformed, transfected, infected and/or injected with a recombinant cloning vehicle and/or DNA sequence encoding isopentenyl monophosphate kinase. Thus, systems and methods are provided for the recombinant expression of the aforementioned recombinant isopentenyl monophosphate kinase that may be used to facilitate its production, isolation and purification in significant amounts. Recombinant isopentenyl monophosphate kinase may be used to obtain expression or enhanced expression of isopentenyl monophosphate kinase in plants in order to enhance the production of isopentenyl monophosphate kinase, or isoprenoids derived therefrom, or may be otherwise employed for the regulation or expression of isopentenyl monophosphate kinase, or the production of its products.

23 Claims, 1 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 1

---------- KWIC ---------

Brief Summary Text - BSTX (5):

In addition, there are examples of cooperation between the cytosolic and plastidial pathways in the biosynthesis of stress-induced and constitutively emitted volatile terpenoids from a variety of plants (Piel, J. et al., Angew.

Chem. Int. Ed., 37:2478-2481 [1998]), and constitutive sesquiterpenes of chamomile (Adam, K.-P. & Zapp, J., Phytochemistry, 48:953-959 [1998]). In mammals, where the DXP pathway is not known to operate, and in plants, the individual biosynthetic steps of the MVA pathway have been well-characterized (Goldstein, J. L. & Brown, M. S., Nature (London), 343:425-430 [1990]; Bach, T. J., Crit. Rev. Biochem. Mol. Biol., 34:107-122 [1999]). However, for the recently discovered DXP pathway, which also occurs in many eubacteria (Rohmer, M., Prog. Drug Res., 50:135-154 [1998]), the biosynthetic sequence leading to the formation of IPP is still incompletely defined (The FIGURE).

Detailed Description Text - DETX (70):

Kinase Assays and Product Identification: Bacterial cells were grown in LB medium supplemented with appropriate antibiotics (ampicillin and chloramphenicol for the expression of the putative peppermint kinase (SEQ ID NO:1), ampicillin for the expression of the putative E. coli kinase (SEQ ID NO:5)) to an OD.sub.600 of 0.2, then treated with either 0.02% arabinose (induction of transgene expression) or 0.02% glucose (repression of transgene expression), and incubated at 20.degree. C. for 20 h. After harvest by centrifugation (1800.times.g, 5 min), the cells were resuspended in 2 ml of assay buffer (100 mM Tris/HCl (pH 7.5) containing 20 mM MgCl.sub.2, 20 mM ATP, and 1 mM DTT), and disrupted by sonication at 0-4.degree. C.; the protein content of the resulting homogenate was determined using the Bio-Rad protein assay. To aliquots containing 400 .mu.g of total protein, 0.79 nmol of the appropriate substrate was added, and the mixture was incubated at 30.degree. C. for 5-60 min. Cell debris was pelleted by centrifugation (13,000 rpm, bench-top centrifuge), protein was removed by filtration through a Nanosep cartridge (10 kDa cut-off; Pall Filtron), and the filtrate was analyzed by reversed-phase ion-pair radio-HPLC using a modification of a previously published method (McCaskill, D. & Croteau, R., Anal. Biochem., 215:142-149 [1993]); column: Adsorbosphere HS C18 (Alltech, 5 .mu.m particle size, 4.6 mm i.d., 250 mm length); solvents: (A) 10 mM tetrabutylammonium acetate (pH 6), (B) 10 mM tetrabutylammonium acetate in 70% aq. methanol (pH 6), (C) 70% aq. methanol; gradient: 100% (A) (hold for 10 min), gradient to 80% (B)/20% (A) (65 min), hold for 10 min, gradient to 100% (C) (10 min), hold for 10 min; flow rate: 1 ml/min.sup.-1 ; retention times: IPP, 72.6 min; DMAPP, 70.4 min; IP, 64.6 min; DMAP, 63.7 min; isopentenol, 32.9 min; dimethylallyl alcohol, 34.6 min; DXP, 49.4 min; DX, 21.2 min; MEP, 46.8 min; MVA, 32.6 min.

US-PAT-NO: 6117641

DOCUMENT-IDENTIFIER: US 6117641 A
See image for Certificate of Correction

TITLE: Assays and reagents for identifying anti-fungal agents and uses related thereto

DATE-ISSUED: September 12, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Berlin; Vivian	Dunstable	MA	N/A	N/A
Levin; David E.	Owens Hills	MD	N/A	N/A
Ohya; Yoshikazu	Tokyo	N/A	N/A	JP

APPL-NO: 08/ 631319

DATE FILED: April 11, 1996

US-CL-CURRENT: 435/7.1, 424/274.1 , 435/15 , 435/7.4

ABSTRACT:

The present invention relates to rapid, reliable and effective assays for screening and identifying pharmaceutically effective compounds that specifically inhibit the biological activity of fungal GTPase proteins, particularly GTPases involved in cell wall integrity, hyphael formation, and/or other cellular functions critical to pathogenesis.

29 Claims, 14 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 14

---------- KWIC ---------

Detailed Description Text - DETX (41):

Still a variety of other formats exist which are amenable to high through put analysis on microtitre plates or the like. The prenylation substrate can be immobilized throughout the reaction, such as by cross-linking to activated polymer, or sequestered to the well walls after the development of the prenylation reaction. In one illustrative embodiment, a Rho-like GTPase, e.g. a fungal Rho1, Rho2, Cdc42 or Rsr1/Bud1, is cross-linked to the polymeric support of the well, the prenylation system set up in that well, and after completion, the well washed and the amount of geranylgeranyl sidechains attached to the immobilized GTPase detected. In another illustrative embodiment, wells of a microtitre plate are coated with streptavidin and contacted with the developed prenylation system under conditions wherein a biotinylated substrate binds to and is sequestered in the wells. Unbound material is washed from the wells, and the level of prenylated target substrate is detected in each well. There are, as evidenced by this specification, a variety of techniques for detecting the level of prenylation of the immobilized substrate. For example, by the use of dansylated (described infra) or radiolabelled geranylgeranyl diphosphaste in the reaction mixture, addition of

appropriate scintillant to the wells will permit detection of the label directly in the microtitre wells. Alternatively, the substrate can be released and detected, for example, by any of those means described above, e.g. by radiolabel, gel electrophoresis, etc. Reversibly bound substrate, such as the biotin-conjugated substrate set out above, is particularly amenable to the latter approach. In other embodiments, only the geranylgeranyl moiety is released for detection. For instance, the thioether linkage of the isoprenoid with the substrate peptide sequence can be cleaved by treatment with methyl iodide. The released geranylgeranyl products can be detected, e.g., by radioactivity, HPLC, or other convenient format.

Detailed Description Text - DETX (122):

The abbreviations used in Example 1 are: PKC, protein kinase C; MAPK, mitogen-activated protein kinase; MEK, MAPK-activating kinase; MEKK, MEK-activating kinase; DAG, diacylglycerol; SRF, serum response factor; JNK, Jun NH.sub.2 -terminal kinase (also known as SAPK, stress-activated protein kinase); PCR, polymerase chain reaction; HA, influenza hemagglutinin; PAGE, polyacrylamide gel electrophoresis; GST, glutathione-S-transferase; PS, phosphatidylserine; PMA, phorbol myristate acetate; GS, 1,3-.beta.-glucan synthase; MBP, myelin basic protein.

US-PAT-NO: 5910433

DOCUMENT-IDENTIFIER: US 5910433 A

TITLE: Keto group-introducing enzyme, DNA coding therefor and method for producing ketocarotenoids

DATE-ISSUED: June 8, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Kajiwara; Susumu	Tokyo	N/A	N/A	JP
Misawa; Norihiko	Kanagawa	N/A	N/A	JP
Kondo; Keiji	Kanagawa	N/A	N/A	JP

APPL-NO: 08/ 632434

DATE FILED: April 23, 1996

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
JP	6-198775	August 23, 1994
JP	6-223798	September 19, 1994
JP	7-047266	March 7, 1995

PCT-DATA:
APPL-NO: PCT/JP95/01640
DATE-FILED: August 18, 1995
PUB-NO: WO96/06172
PUB-DATE: Feb 29, 1996
371-DATE: Apr 23, 1996
102(E)-DATE:Apr 23, 1996

US-CL-CURRENT: 435/148, 435/189 , 435/252.3 , 435/252.33 , 435/320.1 , 435/67 , 435/946 , 536/23.2 , 536/24.3

ABSTRACT:

DNA sequences are described that encode genes for synthesizing ketocarotenoids such as astaxanthin. The DNA sequences, microorganisms containing them and encoded polypeptides are described. Also described are methods to obtain related sequences and to make host cells that contain such sequences. These genes and methods are useful to impart red coloration during culture of fish and crustaceans, as coloring in food, and as antioxidants.

16 Claims, 12 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 12

---------- KWIC ---------

Abstract Text - ABTX (1):
DNA sequences are described that encode genes for synthesizing ketocarotenoids such as astaxanthin. The DNA sequences, microorganisms

containing them and encoded polypeptides are described. Also described are methods to obtain related sequences and to make host cells that contain such sequences. These genes and methods are useful to impart red coloration during culture of fish and crustaceans, as coloring in food, and as antioxidants.

Brief Summary Text - BSTX (10):

Then, it is believed that the acquisition of a group of genes involved in the biosynthesis of astaxanthin would be very useful, because it is possible to render an optimal microorganism with respect of safety as a food and a potential ability to produce astaxanthin, regardless of whether it has an ability to produce astaxanthin or not, the production ability by introducing into the microorganism the group of astaxanthin synthesis genes and expressing them. In this case, there will occur no problem of the mixing of by-products. In addition, by using techniques of the highly advanced genetic engineering, it will not be difficult to increase the amount of astaxanthin production to a level which exceeds the production amount by organic synthesis. As described above, a group of genes to synthesize up to zeaxanthin have already been obtained by the present inventors from the non-photosynthetic bacterium Erwinia uredovora. However, no one has succeeded in obtaining the gene coding for a keto group-introducing enzyme that is necessary for synthesizing astaxanthin, though a number of attempts have been made in many research institute because of the industrial utility of astaxanthin as described above. As to the reasons, it is considered that enzymes located downstream and involved in carotenoid biosynthesis, such as a keto group-introducing enzyme, are membrane proteins and that the purification and measurement of activity of those enzymes have been impossible; therefore, there has been no finding about those enzymes. In particular, as to a keto group-introducing enzyme, not only findings about the enzyme itself but also findings about the gene coding for the enzyme have not been reported at all. Therefore, to date, it has been impossible to produce astaxanthin in a microorganism or the like by using genetic engineering techniques.

Brief Summary Text - BSTX (44):

By using the techniques or methods as described above to introduce a foreign gene into a microorganism, it is possible to introduce into a microorganism a Haematococcus-derived group of ketocarotenoid (including astaxanthin) synthesis genes and express them.

Brief Summary Text - BSTX (46):

Accordingly, by introducing a combination of Erwinia-derived carotenoid synthesis genes with the DNA of the invention (which is typically the Haematococcus-derived carotenoid synthesis gene bkt) into the same microorganism simultaneously, it becomes possible to produce ketocarotenoids such as astaxanthin in all of those microorganisms wherein a gene introduction/expression system has been established. Alternatively, by introducing the DNA of the invention into a microorganism which inherently has carotenoid synthesis genes or a microorganism into which carotenoid synthesis genes have been already introduced, it is also possible to produce ketocarotenoids in the above microorganism. Hereinbelow, the production of various ketocarotenoids by microorganisms will be described.

Brief Summary Text - BSTX (48):

By introducing into a microorganism, such as E. coli, the Erwinia uredovora crtE, crtB, crti and crtY genes necessary for the synthesis of .beta.-carotene and the Haematococcus bkt gene which is a keto group-introducing enzyme gene

and expressing them, it is possible to allow the microorganism to produce canthaxanthin as a final product. Furthermore, by regulating the level of expression of the bkt gene or the like, echinenone which is a synthetic intermediate can also be obtained. For example, in order to produce canthaxanthin and echinenone in E. coli, both a first plasmid (e.g., pACCAR16.DELTA. crtX) obtainable by inserting into an E. coli vector (e.g., pACYC184) a fragment containing the Erwinia uredovora crtE, crtB, crti and crtY genes and a second plasmid [e.g., pHP51 (see FIG. 10)] obtainable by inserting into an E. coli vector (e.g., pBluescript II KS+) a fragment containing the Haematococcus bkt gene are introduced into E. coli (e.g., JM101). The resultant E. coli is cultured in LB medium, 2YT medium or the like containing ampicillin and chloramphenicol under culture conditions at 30-37.degree. C. until the stationary phase. Then, cells are harvested and carotenoid pigments are extracted by using an organic solvent such as acetone. Canthaxanthin and echinenone may be contained in the carotenoid pigments thus obtained.

Detailed Description Text - DETX (5):

The Haematococcus pluvialis used for obtaining genes is the NIES-144 strain registered at the Foundation Global Environmental Forum. H. pluvialis was cultured for 4 days in basal medium (yeast extract 0.2%; sodium acetate 0.12%; L-asparagine 0.04%; magnesium chloride.6H.sub.2 O 0.02%; iron(II) sulfate.7H.sub.2 O 0.001%; calcium chloride.2H.sub.2 O 0.002%) at 20.degree. C. under 12 hr light/12 hr dark cycles (20 .mu.E/m.sup.2 .multidot.s). Further, for the induction of astaxanthin synthesis in H. pluvialis, acetic acid was added to the H. pluvialis NIES-144 strain to a final concentration of 45 mM and iron(II) sulfate. 7H.sub.2 O to a final concentration of 450 .mu.m, and the strain was cultured at 20.degree. C. at a photointensity of 125 .mu.E/m.sup.2 .multidot.s for about 12 hours to thereby induce the formation of cysts.

Detailed Description Text - DETX (27):

By subjecting plasmid pCAR16 which contains all of the Erwinia uredovora carotenoid synthesis genes (crtE, crtX, crtY, crtI and crtB) other than crtZ (see Misawa, N., Nakagawa, M., Kobayashi, K., Yamano, S., Izawa, Y., Nakamura, K. and Harashima, K., "Elucidation of the Erwinia uredovora carotenoid biosynthetic pathway by functional analysis of gene products expressed in Escherichia coli", J. Bacteriol., 172, pp. 6704-6712, 1990; and Japanese Unexamined Patent Publication No. 3-58786) to BstEII digestion, Klenow enzyme treatment and a ligase reaction, the crtX gene was deactivated by a frameshift. Then, a 6.0 kb Asp718(KpnI)-EcoRI fragment was cut out which contains the crtE, crtY, crtI and crtB genes necessary for .beta.-carotene production. This fragment was inserted into the EcoRV site of E. coli vector pACYC184 (obtained from ATCC 37033) to thereby obtain the plasmid of interest (designated as pACCAR16.DELTA. crtX). The E. coli carrying this pACCAR16.DELTA. crtX exhibits chloramphenicol resistance and can produce .beta.-carotene.

Detailed Description Text - DETX (52):

On the other hand, according to the researches of the present inventors using carotenoid synthesis genes from the bacteria Erwinia present in plants and the photosynthetic bacteria Rhodobacter, it has become clear that, generally, a carotenoid biosynthesis enzyme recognizes only one half of the carotenoid molecule which is a substrate and acts on it. For example, crtY which is a lycopene ring formation enzyme gene recognizes by one half of the lycopene molecule and makes the ring formation. Therefore, by using the phytoene desaturase gene crtI from Rhodobacter, it is possible to allow E. coli to produce neurosporene instead of lycopene. And when the produced

neurosporene is treated with the erwinia-derived crtY, the crtY gene product recognizes only the half structure of a neurosporene molecule which is common with lycopene and, as a result, .beta.-zeacarotene is produced which is circulized by half (see Linden, H., Misawa, N., Chamovitz, D., Pecker, I., Hirschberg, J. and Sandmann, G., "Functional complementation in Escherichia coli of different phytoene desaturase genes and analysis of accumulated carotenes", Z. Naturforsch., 46c, pp. 1045-1051, 1991). In addition, in the present invention also, when .beta.-carotene was treated with BKT, first echinenone is synthesized wherein one keto group is introduced, and when zeaxanthin is treated with BKT, first 4-ketozeaxanthin is synthesized wherein one keto group is introduced. This can be considered that BKT recognizes one half of a substrate molecule and introduces a keto group at position 4. On the other hand, the E. coli carrying the Erwinia-derived crtE, crtB, crtI, crtY and crtZ genes produces zeaxanthin as described above, but .beta.-cryptoxanthin wherein one hydroxyl group is introduced into .beta.-carotene can also be detected in the products as an intermediary metabolite. This means that, if BKT is present there, 3'-hydroxyechinenone and 3-hydroxyechinenone can be produced with the .beta.-cryptoxanthin as a substrate. In addition, it can be also considered that BKT further acts on these substances produced to thereby synthesize phoenicoxanthin. This time, we have not achieved the identification of these substances in cultures, because under the conditions employed for this time it seems that these substances are present only in extremely small amounts. In fact, in the typical astaxanthin-producing microorganism Phaffia rhodozyma which is comparable with Haematococcus, 3-hydroxyechinenone and phoenicoxanthin are detected as intermediary metabolites of astaxanthin (Andrewes, A. G., Phaff, H. J. and Starr, M. P., "Carotenoids of Phaffia rhodozyma, a redpigmented fermenting yeast", Phytochemistry, 15, pp. 1003-1007, 1976). From so far described, it is possible to consider that there are the minor metabolic pathways shown in FIG. 9 other than the major astaxanthin metabolic pathway shown in FIG. 8.

Other Reference Publication - OREF (11):
"Functional Complementation in Escherichia Coli of Different Phytoene Desaturase Genes and Analysis of Accumulated Carotenes", Linden et al., (1991) Z. Naturforsch, 46e, 1045-1051.

US-PAT-NO: 5837815

DOCUMENT-IDENTIFIER: US 5837815 A

TITLE: PYK2 related polypeptide products

DATE-ISSUED: November 17, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Lev; Sima	San Francisco	CA	N/A	N/A
Schlessinger; Joseph	New York	NY	N/A	N/A

APPL-NO: 08/ 460626

DATE FILED: June 2, 1995

PARENT-CASE:

RELATED APPLICATION

The present application is a continuation-in-part application of U.S. application Ser. No. 08/357,642, filed Dec. 15, 1994, incorporated herein by reference in its entirety, including any drawings.

US-CL-CURRENT: 530/350, 435/69.1 , 530/412

ABSTRACT:

The present invention features a method for treatment of an organism having a disease or condition characterized by an abnormality in a signal transduction pathway, wherein the signal transduction pathway include a PYK2 protein. The invention also features methods for diagnosing such diseases and for screening for agents that will be useful in treating such diseases. The invention also features purified and/or isolated nucleic acid encoding a PYK2 protein.

8 Claims, 4 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 4

---------- KWIC ---------

Detailed Description Text - DETX (5):

PYK2 is activated by extracellular signals that lead to calcium influx or calcium release from internal stores. PYK2 is phosphorylated on tyrosine residues in response to a variety of external stimuli that cause membrane depolarization and Ca.sup.+2 influx such as the activation of the nicotinic acetylcholine receptor. Tyrosine phosphorylation of PYK2 is also stimulated by the neuropeptide Bradykinin that activates a G-protein coupled receptor as well as by Phorbol myristate acetate (PMA). Experiments in transfected cells and in Xenopus oocytes, microinjected with PYK2 mRNA, indicate that activation of PYK2 can lead to tyrosine phosphorylation of a delayed rectifier-type potassium channel protein and to suppression of potassium currents via this channel. These results suggest a novel mechanism by which a non-receptor tyrosine

kinases, in the nervous system, can be both activated by and can modulate the action of ion-channel proteins.

Detailed Description Text - DETX (291):

Incubation of PC12 cells with phorbol myristate acetate (PMA) induced tyrosine phosphorylation of PYK2, suggesting that tyrosine phosphorylation of PYK2 could also be mediated via protein kinase C (PKC) activation. To determine whether bradykinin-induced phosphorylation of PYK2 is mediated via PKC, the cells were treated with bradykinin or PMA following down-regulation of PMA-sensitive PKC isozymes by prolonged treatment with PMA. Prolonged treatment with PMA completely abolished the effect of PMA, but had only a minor effect on bradykinin-stimulated tyrosine phosphorylation of PYK2. These results suggest that tyrosine phosphorylation of PYK2 can be induced by PKC-independent and by PKC-dependents mechanism.

Other Reference Publication - OREF (66):

Inouye and Inouye, "Up-promotor mutations in the lpp gene of Escherichia coli," Nucleic Acids Research 13(9):3100-3111 (1985).

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	6162	isoprenoid$1 or lycopene$1 or carotene$1 or astaxanthin$1 or phytoene$1 or isopentyl adj diphosphate or ipp	USPAT	OR	OFF	2004/02/16 15:03
L2	147	acetyl adj phosphate or acetylphosphate	USPAT	OR	OFF	2004/02/16 15:05
L3	219	acetyl adj phosphate or acetylphosphate	US-PGPUB; USPAT	OR	OFF	2004/02/16 15:05
L4	0	1 near4 (gene$1 or sequence$1) and (L2 or L3)	USPAT	OR	OFF	2004/02/16 15:07
L5	306498	acetate	USPAT	OR	OFF	2004/02/16 15:06
L6	122	1 near4 (gene$1 or sequence$1) and L5	US-PGPUB; USPAT	OR	OFF	2004/02/16 15:13
L7	0	1 near4 (gene$1 or sequence$1) and L2	US-PGPUB; USPAT	OR	OFF	2004/02/16 15:22
L8	1278906	induc$8 or regulat$8 or activat$8 or modulat$8	USPAT	OR	OFF	2004/02/16 15:13
L9	12	1 near4 (gene$1 or sequence$1) and (L5 same L8)	US-PGPUB; USPAT	OR	OFF	2004/02/16 15:13
L10	721	1 same (2 or 5)	US-PGPUB; USPAT	ADJ	OFF	2004/02/16 15:22
L11	2	1 near4 (gene$1 or sequence$1) same (2 or 5)	US-PGPUB; USPAT	OR	OFF	2004/02/16 15:22

US-PAT-NO: 5705624

DOCUMENT-IDENTIFIER: US 5705624 A

TITLE: DNA sequences encoding enzymes useful in phytoene biosynthesis

DATE-ISSUED: January 6, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Fitzmaurice; Wayne Paul	Vacaville	CA	95687	N/A
Hellmann; Gary Mark	Clemmons	NC	27012	N/A
Grill; Laurence Kay	Vacaville	CA	95688	N/A
Kumagai; Monto Hiroshi	Davis	CA	95616	N/A
della-Cioppa; Guy Richard	Vacaville	CA	95688	N/A

APPL-NO: 08/ 579667

DATE FILED: December 27, 1995

US-CL-CURRENT: 536/23.2, 435/183 , 536/23.6

ABSTRACT:

DNA sequences isolated from Nicotiana species (e.g., Nicotiana tabacum and Nicotiana benthamiana) and encoding polypeptides having enzymatic activity for producing phytoene, and the polypeptides encoded, are provided.

4 Claims, 2 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 2

---------- KWIC ---------

Detailed Description Text - DETX (60):

Hybridization and detection of phytoene synthase homologous nucleotide sequences were performed using a chemiluminescent system. The membrane was prehybridized for 1 hr at 60.degree. C. in 10 ml hybridization solution (5.times. SSC, 1.times. Blocking solution 0.1% N-laurylsarcosinate, 0.02% sodium dodecylsulfate (SDS); 10.times. Blocking solution is 10% (w/v) Blocking Reagent (Boehringer Mannheim Biochemicals, Indianapolis, Ind.) in 100 mM maleic acid at pH 7.5, 150 mM NaCl). Incubations were in a rotating bottle hybridization incubator. Heat-denatured (5 min at 100.degree. C.) probe was added to the prehybridization mixture at a final concentration of 1.25 ng/ml, and incubation of the resulting mixture was continued 4-16 hr at 60.degree. C. The membrane was washed twice for 5 min in 2.times. SSC, 0.1% SDS at room temperature, and twice for 15 min in 0.1.times. SSC, 0.1% SDS at 60.degree. C. For chemiluminescent detection the membrane was rinsed in 100 mM maleic acid, 150 mM NaCl, and incubated for 30 min at room temperature in 1.times. Blocking solution. Alkaline phosphatase-conjugated anti-digoxigenin Fab fragments were added (1:10,000 dilution), and incubation was continued for 30 min at room temperature. The membrane was washed twice for 15 min in 100 mM maleic acid, 150 mM NaCl, at room temperature. The membrane was equilibrated

for 2 min in 100 mM Tris-HCl (pH 9.5), 100 mM NaCl, 50 mM MgCl.sub.2. The membrane was placed on a sheet of clear acetate film and Lumi-Phos 530 (Boehringer Mannheim Biochemicals, Indianapolis, Ind.) was added on the DNA side of the membrane. Another sheet of acetate film was used to cover the membrane, and the membrane was exposed to X-ray film. Positive signals on the X-ray film indicated the presence of PCR products containing phytoene synthase homologous gene sequences.

US-PAT-NO: 5539093

DOCUMENT-IDENTIFIER: US 5539093 A

TITLE: DNA sequences encoding enzymes useful in carotenoid biosynthesis

DATE-ISSUED: July 23, 1996

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Fitzmaurice; Wayne P.	Clemmons	NC	27012	N/A
Hellmann; Gary M.	Clemmons	NC	27012	N/A
Grill; Laurence K.	Vacaville	CA	95688	N/A
Kumagai; Monto H.	Davis	CA	95616	N/A
della-Cioppa; Guy R.	Vacaville	CA	95688	N/A

APPL-NO: 08/ 261086

DATE FILED: June 16, 1994

US-CL-CURRENT: 536/23.2, 435/189

ABSTRACT:

DNA sequences isolated from Nicotiana species (e.g., Nicotiana benthamiana and Nicotiana tabacum) have SEQ ID NOS: 1, 3, 5 and 7. The DNA sequences encode polypeptides having enzymatic activity for producing zeta-carotene. The polypeptides are referred to as phytoene desaturase.

13 Claims, 1 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 1

---------- KWIC ---------

Detailed Description Text - DETX (35):

Hybridization and detection of phytoene desaturase homologous sequences were performed using a chemiluminescent system. The membrane was prehybridized for 1 hr at 60.degree. C. in 10 ml hybridization solution (5X SSC., 1X Blocking solution 0.1% N-laurylsarcosinate, 0.02% sodium dodecylsulfate (SDS); 10X Blocking solution is 10% (w/v) Blocking Reagent (Boehringer Mannhelm Biochemicals, Indianapolis, Ind.) in 100 mM maleic acid at pH 7.5, 150 mM NaCl). Incubations were in a rotating bottle hybridization incubator. Heat-denatured (5 min at 100.degree. C.) probe was added to the prehybridization mixture at a final concentration of 1.25 ng/ml, and incubation of the resulting mixture was continued 4-16 hr at 60.degree. C. The membrane was washed twice for 5 min in 2X SSC., 0.1% SDS at room temperature, and twice for 15 min in 0.1X SSC, 0.1% SDS at 60.degree. C. For chemiluminescent detection the membrane was rinsed in 100 mM maleic acid, 150 mM NaCl, and incubated for 30 min at room temperature in 1X Blocking solution. Alkaline phosphatase-conjugated anti-digoxigenin Fab fragments were added (1:10,000 dilution), and incubation was continued for 30 min at room temperature. The membrane was washed twice for 15 min in 100 mM maleic acid, 150 mM NaCl, at

room temperature. The membrane was equilibrated for 2 min in 100 mM Tris-HCl (pH 9.5), 100 mM NaCl, 50 mM MgCl.sub.2. The membrane was placed on a sheet of clear acetate film and Lumi-Phos 530 (Boehringer Mannhelm Biochemicals, Indianapolis, Ind.) was added on the DNA side of the membrane. Another sheet of acetate film was used to cover the membrane, and the membrane was exposed to X-ray film. Positive signals on the X-ray film indicated the presence of PCR products containing phytoene desaturase homologous sequence.

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	6162	isoprenoid$1 or lycopene$1 or carotene$1 or astaxanthin$1 or phytoene$1 or isopentyl adj diphosphate or ipp	USPAT	OR	OFF	2004/02/16 15:03
L2	147	acetyl adj phosphate or acetylphosphate	USPAT	OR	OFF	2004/02/16 15:05
L3	219	acetyl adj phosphate or acetylphosphate	US-PGPUB; USPAT	OR	OFF	2004/02/16 15:05
L4	0	1 near4 (gene$1 or sequence$1) and (L2 or L3)	USPAT	OR	OFF	2004/02/16 15:07
L5	306498	acetate	USPAT	OR	OFF	2004/02/16 15:06
L6	122	1 near4 (gene$1 or sequence$1) and L5	US-PGPUB; USPAT	OR	OFF	2004/02/16 15:13
L7	0	1 near4 (gene$1 or sequence$1) and L2	US-PGPUB; USPAT	OR	OFF	2004/02/16 15:22
L8	1278906	induc$8 or regulat$8 or activat$8 or modulat$8	USPAT	OR	OFF	2004/02/16 15:13
L9	12	1 near4 (gene$1 or sequence$1) and (L5 same L8)	US-PGPUB; USPAT	OR	OFF	2004/02/16 15:13
L10	721	1 same (2 or 5)	US-PGPUB; USPAT	ADJ	OFF	2004/02/16 15:22
L11	2	1 near4 (gene$1 or sequence$1) same (2 or 5)	US-PGPUB; USPAT	OR	OFF	2004/02/16 15:24
L12	6	6 and carbon adj (flux or flow)	US-PGPUB; USPAT	OR	OFF	2004/02/16 15:25

US-PAT-NO: 6689601

DOCUMENT-IDENTIFIER: US 6689601 B2

TITLE: High growth methanotropic bacterial strain

DATE-ISSUED: February 10, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Koffas; Mattheos	Wilmington	DE	N/A	N/A
Odom; James M.	Kennett Square	PA	N/A	N/A
Schenzle; Andreas	Zurich	N/A	N/A	CH

APPL-NO: 09/ 934868

DATE FILED: August 22, 2001

PARENT-CASE:

This application claims the benefit of U.S. Provisional Application No. 60/229,858 filed Sep. 1, 2000.

US-CL-CURRENT: 435/247, 435/232 , 435/248 , 435/250 , 435/252.1 , 435/71.1 , 536/24.1

ABSTRACT:

A high growth methanotrophic bacterial strain capable of growth on a C1 carbon substrate has been isolated and characterized. The strain has the unique ability to utilize both methane and methanol as a sole carbon source and has been demonstrated to possess a functional Embden-Meyerhof carbon flux pathway. The possession of this pathway conveys an energetic advantage to the strain, making it particularly suitable as a production platform for the production of biomass from a C1 carbon source.

14 Claims, 6 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 6

---------- KWIC ---------

Abstract Text - ABTX (1):
A high growth methanotrophic bacterial strain capable of growth on a C1 carbon substrate has been isolated and characterized. The strain has the unique ability to utilize both methane and methanol as a sole carbon source and has been demonstrated to possess a functional Embden-Meyerhof carbon flux pathway. The possession of this pathway conveys an energetic advantage to the strain, making it particularly suitable as a production platform for the production of biomass from a C1 carbon source.

Brief Summary Text - BSTX (7):
The conversion of C1 compounds to complex molecules with C--C bonds is a

difficult and capital intensive process by traditional chemical synthetic routes. Traditionally, methane is first converted to synthesis gas (mixtures of hydrogen, carbon monoxide and carbon dioxide), which is then used to produce other small molecular weight industrial precursors. Typically these are "commodity" type chemicals such as acetate, formaldehyde, or methanol. The basic problem is activation of the methane molecule which is thermodynamically very difficult to achieve by chemical means. "Activation" refers to the process of making the chemically unreactive methane molecule more reactive.

Brief Summary Text - BSTX (11):

As noted above, methanotrophic bacteria possess the potential to be commercially effective production platforms for materials such as single cell protein, exopolysaccharides, and long chain carbon molecules such as isoprenoids and carotenoid pigments. The usefulness of methanotrophs for production of a larger range of chemicals is constrained however, by several limitations including, relatively slow growth rates of methanotrophs, limited ability to tolerate methanol as an alternative substrate to methane, difficulty in genetic engineering, poor understanding of the roles of multiple carbon assimilation pathways present in methanotrophs, and potentially high costs due to the oxygen demand of fully saturated substrates such as methane. The problem to be solved therefore is to develop a fast-growing, high yielding methanotroph capable of receiving foreign genes via standard genetic procedures. Full and rapid resolution of central carbon pathways is essential for enabling pathway engineering and carbon flux management for new products.

Brief Summary Text - BSTX (21):

In one embodiment the present strain may optionally contain other carbon flux genes encoding polypeptides selected from the group consisting of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18 and 20.

Brief Summary Text - BSTX (24):

In a more specific embodiment the present strain may comprise genes encoding isoprenoid synthesizing enzymes where the enzymes are selected from the group consisting of SEQ ID NO:62, 64, 66, 68, 70, 72, 74, 86, and 78.

Detailed Description Text - DETX (5):

The present invention describes the isolation and characterization of a high growth methanotrophic bacterial strain useful for the production of biomass including proteins, carbohydrates and pigments. The present strain is typed by 16sRNA as a Methylomonas sp. and is referred to herein as Methylomonas 16a. In addition, the strain may be useful for the production of mixtures of proteins, carbohydrates and pigments for the purpose of generating animal feeds. The strain possesses the advantage of an active Embden-Meyerhof carbon flux pathway having a pyrophosphate dependent phosphofructokinase gene, which conveys certain energetic advantages to the strain as a production platform for various materials and biomass. Additionally the strain naturally possesses an active isoprenoid pathway for the generation of pigments indigenous to the strain. In this disclosure, a number of terms and abbreviations are used. The following definitions are provided.

Detailed Description Text - DETX (11):

The term "high growth methanotrophic bacterial strain" refers to a bacterium capable of growth with methane or methanol as a sole carbon and energy source and which possesses a functional Embden-Meyerhof carbon flux pathway resulting

in a yield of cell mass per gram of C1 substrate metabolized. The specific "high growth methanotrophic bacterial strain" described herein is referred to as "Methylomonas 16a" or "16a", which terms are used interchangeably.

Detailed Description Text - DETX (30):

The present invention provides a unique methanotrophic bacterial strain, useful for the production of a variety materials from C1 carbon sources such as methane and methanol. The strain is referred to herein as Methylomonas 16a, and is characterized by rapid doubling time, high yield and the presence of genes encoding both the Entner-Douderoff carbon pathway as well as the Embden-Meyerhof pathway, allowing for versatility in carbon flux management and higher efficiency of carbon incorporation. The strain has been shown to produce a variety of food and feed products such as single cell protein, exopolysaccharide and starch. The strain has particularly high value in the production of food and feed materials as it is possible to manipulate the various concentrations of protein, carbohydrate and starch all within the same organism. This capability will permit strains to be uniquely tailored for individual specific food and feed applications. Additionally the strain has demonstrated utility in the production of terpenoid and carotenoid compounds, useful as pigments and as monomers in polymeric materials.

Detailed Description Text - DETX (39):

A particularly novel and useful feature of the Embden-Meyerhof pathway in strain 16a is that the key phosphofructokinase step is pyrophosphate dependent instead of ATP dependent. This feature adds to the energy yield of the pathway by using pyrophosphate instead of ATP (Example 6). Because of its significance in providing an energetic advantage to the strain, this gene in the carbon flux pathway is considered diagnostic for the present strain.

Detailed Description Text - DETX (45):

Accordingly the present invention provides a Methylomonas having two distinct carbon flux pathways, comprising genes and gene products as set forth in SEQ ID NO:1-20, and encoding both a pyrophosphate dependent phosphofructokinase pyrophosphate and a keto-deoxy phosphogluconate (KDPG) aldolase. Comparison of the KDPG aldolase gene sequence (SEQ ID NO:19) and deduced amino acid sequence (SEQ ID NO:20) to public databases reveals that the most similar known sequences is about 59% identical to the amino acid sequence of reported herein over a length of 212 amino acid using a Smith-Waterman alignment algorithm (W. R. Pearson, supra). More preferred amino acid fragments are at least about 80%-90% identical to the sequences herein. Most preferred are nucleic acid fragments that are at least 95% identical to the amino acid fragments reported herein. Similarly, preferred KDPG aldolase encoding nucleic acid sequences corresponding to the instant gene are those encoding active proteins and which are at least 80% identical to the nucleic acid sequences of reported herein. More preferred KDPG aldolase nucleic acid fragments are at least 90% identical to the sequences herein. Most preferred are KDPG aldolase nucleic acid fragments that are at least 95% identical to the nucleic acid fragments reported herein.

Detailed Description Text - DETX (47):

In addition to the pyrophosphate dependent phosphofructokinase enzyme and keto-deoxy phosphogluconate aldolase enzyme, the strain comprises other carbon flux genes including an FBP aldolase, phosphoglucomutase, pyrophosphate dependent phosphofructokinase pyrophosphate, 6-Phosphogluconate dehydratase, and a glucose-6 phosphate-1 dehydrogenase. The phosphoglucomutase is

responsible for the interconversion of glucose-6-phosphate to glucose-1-phosphate, which feeds into either the Entner-Douderoff or Embden-Meyerhof carbon flux pathways. As shown in FIG. 3, fructose-6-phosphate may be convert to either glucose-6-phosphase by glucose phosphate isomerase (Entner-Douderoff) or to fructose-1,6-bisphosphate (FBP) by a phosphofructokinase (Embden-Meyerhof). Following the Embden-Meyerhof pathway, FBP is then taken to two three-carbon moieties (dihydroxyacetone and 3-phosphoglyceraldehyde) by the FBP aldolase. Returning to the Entner-Douderoff system, glucose-6-phosphate is taken to 6-phosphogluconate by a glucose-6-phosphate dehydrogenase which is subsequently taken to 2-keto-3-deoxy-6-phosphogluconate (KDPG) by a 6 phosphogluconate dehydratase. The KDPG is then converted to two three-carbon moieties (pyruvate and 3-phosphoglyceraldehyde) by a KDPG aldolase. Thus the Embden-Meyerhof and Entner-Douderoff pathways are rejoined at the level of 3-phosphoglyceraldehyde.

Detailed Description Text - DETX (52):

Growth Characteristics: The presence of the above mentioned carbon flux characteristics was previously unknown in methanotrophic bacteria and may explain the rapid growth rate and the increased carbon conversion efficiency of this strains and other strains possessing this pathway, relative to strain that do not have this pathway. The present Methylomonas 16a has been shown to grow on methane with a doubling time of only 2.5 h. This is a very high growth rate and is an obvious advantage for commercial use as well as for the genetic manipulations performed in development of the strain. Additionally, Methylomonas has no requirement for organic growth factors such as yeast extract or other costly fermentation additives. The strain requires only methane or methanol, inorganic minerals, oxygen and water for optimum growth, giving the present strain an advantage for large scale growth at low cost.

Detailed Description Text - DETX (57):

Pigment and Terpenoid Production: The present Methylomonas strain is useful for the production of a variety of pigments and particularly the isoprenoid pigments. This class of pigments are known to have strong light absorbing properties and are derived from the head to tail condensation of 5, 10, 15, 20, 25, 30 or 40 carbon isoprene chains. One specific pigment identified in the present strain is a C-30 carotenoid. The content of this pigment is very high in the cell and is indicative of naturally high carbon flow through the isoprenoid pathway. This aspect provides the basis for viewing the isoprenoid pathway as a "backbone production pathway" for isoprenoid-derived products. It is contemplated for example that high value carotenoids such as astaxanthin, .beta.-carotene, canthaxanthin, and lutein may be produced by the instant organism.

Detailed Description Text - DETX (68):

Further down in the isoprenoid biosynthesis pathway, more genes are involved in the synthesis of specific isoprenoids. As an example, the crtN gene that was found in Heliobacillus mobilis (Proc. Natl. Acad. Sci. USA 95:14851-14856 (1998)) encodes a diapophytoene dehydrogenase that is a part of the carotenoid biosynthesis pathway.

Detailed Description Text - DETX (69):

Although some of the genes involved in isoprenoid pathways are well known, the presence of genes involved in the isoprenoid pathway of Methylomonas sp. is rare. It is surprising therefore to find all of the above mentioned genes in the present strain (SEQ ID NO:61-SEQ ID NO:78). Tgus suggests that the

present strain will be useful for the production of a variety of terpenoids. Accordingly the invention provides a Methylomonas strain having the genes and gene products as set forth in SEQ ID NO:61-SEQ ID NO:78, encoding a D-1-deoxyxylulose-5-phosphate synthase, a D-1-deoxyxylulose-5-phosphate reductoisomerase, 2C-methyl-d-erythritol 2,4-cyclodiphosphate synthase, a 2C-methyl-d-erythritol cytidylyltransferase, a CTP synthase, a Geranyltranstransferase (also farnesyl-diphosphate synthase), a 4-diphosphocytidyl-2-C-methylerythritol kinase, and a diapophytoene dehydrogenase.

Detailed Description Text - DETX (70):

Production of Single Cell Protein: The present strain is useful for the production of single cell protein (SCP) which has value in the food and feed industries. Methods for the use of methanotrophs as production platforms for the production of SCP are well known in the art (see for example U.S. Pat. No. 4,795,708; Shojaosadati et al., Amirkabir (1996), 8(30), 33-41). The present strain is well suited for this application due to its advantages in carbon flux and reduced oxygen consumption in the presence of a nitrogen source. The strain is well suited for the production of single cell protein under either aerobic or anaerobic conditions.

Detailed Description Text - DETX (91):

Carotenoid pigments play a role in terms of providing coloration for many aquatic fish and crustacean species as well as providing antioxidant benefit. (Nelis H. J., De Leenheer 1991. J. Appl. Bacteriol. 70:181-191). Methylomonas 16a, unlike many commercially utilized methanotrophs (i.e. Methylococcus capsulatus) has a natural carotenoid pigment production pathway which produces high levels of a pink pigment that is similar, but not structurally identical, with such high value carotenoids as astaxanthin. Modification of this pathway by addition of genes involved in the final steps of astaxanthin synthesis or other high value carotenoids will result in the ability of this strain to produce these carotenoids. In this way Methylomonas 16a will be uniquely useful as an animal feed production strain in which the ratios of protein/carbohydrate/pigments may be tailored to suit particular nutritional needs. In this way, Methylomonas may be utilized as a way to deliver higher value components to other sources of plant protein or carbohydrate and thus circumvent the problem of genetic engineering of these plants for the higher value traits.

Detailed Description Text - DETX (98):

Within the context of the present invention it may be useful to modulate the expression of the identified biosynthetic pathways. For example, it has been noted that the present Methylomonas 16a comprises genes encoding both the Entner-Douderoff and Embden-Meyerhof carbon flux pathways. Because the Embden-Meyerhof pathway is more energy efficient it may be desirable to over-express the genes in this pathway. Additionally, it is likely that the Entner-Douderoff pathway is a competitive pathway and inhibition of this pathway may lead to increased energy efficiency in the Embden-Meyerhof system. This might be accomplished by selectively using the above described methods of gene down regulation on the sequence encoding the keto-deoxy phosphogluconate aldolase (SEQ ID NO:9) or any of the other members of the Entner-Douderoff system and upregulating the gene encoding the fructose bisphosphatase aldolase of the Embden-Meyerhof system (SEQ ID NO:5 OR 7). In this fashion the carbon flux in the present Methylomonas 16a may be optimized. Additionally, where the present strain has been engineered to produce specific organic materials such as aromatics for monomer production, optimization of the carbon flux pathway

will lead to increased yields of these materials.

Detailed Description Text - DETX (99):
In a similar fashion the genes encoding the key enzymes involved in isoprenoid or pigment synthesis may be modulated. For example, the present invention provides a number of genes encoding key enzymes in the terpenoid pathway leading to the production of pigments and smaller isoprenoid compounds. The isolated genes include the dxs and dsr genes, the ispA, D, E, F, and G genes, the pyrg gene, and crtN genes. In particular it may be useful to up-regulate the initial condensation of 3-carbon molecules (pyruvate and C1 aldehyde group, D-glyceraldehyde 3-Phosphate), to yield the 5-carbon compound (D-1-deoxyxylulose-5-phosphate) mediated by the dxs gene. Alternatively, if it is desired to produce a specific non-pigmented isoprenoid, it may be desirable to disrupt various genes at the downstream end of the pathway. For example, it may be desirable to use gene disruption or antisense inhibition of the crtN gene (known to encode diapophytoene dehydrogenase) if a smaller, upstream terpenoid is the desired product of the pathway.

Detailed Description Text - DETX (122):
A number of genes encoding specific identifying enzymes were isolated and sequenced from Methylomonas 16a. These include distinguishing genes found in the Entner-Douderoff carbon flux pathway the Embden-Meyerhof carbon flux pathway, genes encoding a denitrification pathway, genes encoding an isoprenoid synthesis pathway, and genes encoding a pathway for the synthesis of exopolysaccharides. These genes were sequenced and functionally characterized by comparison of their respective sequences to information in public nucleic acid and protein databases according to the following procedures.

Detailed Description Text - DETX (125):
Library construction 200 to 500 .mu.g of chromosomal DNA was resuspended in a solution of 300 mM sodium acetate, 10 mM tris-HCl, 1 mM Na-EDTA, and 30% glycerol, and sheared at 12 psi for 60 sec in an Aeromist Downdraft Nebulizer chamber (IBI Medical products, Chicago, Ill.). The DNA was precipitated, resuspended and treated with Bal31 nuclease. After size fractionation, a fraction (2.0 kb, or 5.0 kb) was excised, cleaned and a two-step ligation procedure was used to produce a high titer library with greater than 99% single inserts.

Detailed Description Paragraph Table - DETL (1):
SEQ ID SEQ ID Description Nucleic acid Peptide Phosphoglucomutase; carbon Flux 1 2 Glucose 6 phosphate 3 4 isomerase; Carbon flux Phosphofructokinase pyrophosphate 5 6 dependent; Carbon Flux 6-Phosphogluconate 7 8 dehydratase; Carbon flux Glucose 6 phosphate 1 9 10 dehydrogenase; Carbon Flux Transaldolase; Carbon Flux 11 12 Transaldolase; Carbon Flux 13 14 Fructose bisphosphate 15 16 aldoslase; Carbon Flux Fructose bisphosphate 17 18 aldoslase; Carbon Flux KHG/KDPG Aldolase; Carbon Flux 19 20 ugp: Exopolysaccharaide 21 22 gumD: Exopolysaccharaide 23 24 wza: Exopolysaccharaide 25 26 epsB: Exopolysaccharaide 27 28 epsM: Exopolysaccharaide 30 20 waaE: Exopolysaccharaide 31 32 epsV: Exopolysaccharaide 33 34 gumH: Exopolysaccharaide 35 36 glycosyl 37 38 transferase: Exopolysaccharaide nirF: Denitrification 39 40 nirD: Denitrification 41 42 nirL: Denitrification 43 44 nirG: Denitrification 45 46 nirH: Denitrification 47 48 nirJ: Denitrification 49 50 nasA: Denitrification 51 52 norC: Denitrification 53 54 norB: Denitrification 55 56 norZ: Denitrification 57 58 norS: Denitrification 59 60 dxs: Terpenoid synthesis 61

62 dxr: Terpenoid synthesis 63 64 ispF: Terpenoid synthesis 65 66 ispD: Terpenoid synthesis 67 68 pyrG: Terpenoid synthesis 69 70 IspA: Terpenoid synthesis 71 72 IspE: Terpenoid synthesis 73 74 crtN: Terpenoid synthesis 75 76 crtN1: Terpenoid synthesis 77 78 Particulate monooxygenase 79 80 16sRNA for Methylomonas 16a 81 --

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TITLE: Production of farnesol and geranylgeraniol

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INVENTOR-INFORMATION:

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PARENT-CASE:

CROSS REFERENCE TO RELATED APPLICATIONS

The present application is a continuation of U.S. patent application Ser. No. 09/350,275, filed Jul. 6, 1999, entitled "PRODUCTION OF FARNESOL AND GERANYLGERANIOL," now U.S. Pat. No. 6,531,303 which claims priority from U.S. Provisional Application Serial No. 60/091,964, filed Jul. 6, 1998, entitled "PRODUCTION OF FARNESOL AND GERANYLGERANIOL," both of which are incorporated herein by this reference.

US-CL-CURRENT: 435/155, 435/193 , 435/252.33 , 435/254.21 , 435/69.2

ABSTRACT:

The invention provides a biological method of producing farnesol or geranylgeraniol.

24 Claims, 11 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 11

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Detailed Description Text - DETX (8):

Suitable biological systems for producing farnesol and GG include prokaryotic and eukaryotic cell cultures and cell-free systems. Preferred biological systems include fungal, bacterial and microalgal systems. More preferred biological systems are fungal cell cultures, more preferably a yeast cell culture, and most preferably a Saccharomyces cerevisiae cell culture. Fungi are preferred since they have a long history of use in industrial processes and can be manipulated by both classical microbiological and genetic engineering techniques. Yeast, in particular, are well-characterized genetically. Indeed, the entire genome of S. cerevisiae has been sequenced, and the genes coding for enzymes in the isoprenoid pathway have already been cloned. Also, S. cerevisiae grows to high cell densities, and amounts of

squalene and ergosterol (see FIG. 1) up to 16% of cell dry weight have been reported in genetically-engineered strains. For a recent review of the isoprenoid pathway in yeast, see Parks and Casey, Annu. Rev. Microbiol. 49:95-116 (1995).

Detailed Description Text - DETX (18):

A further embodiment of the present invention is the use of a microorganism which has been genetically modified to increase the action of HMG-CoA reductase. It should be noted that reference to increasing the action of HMG-CoA reductase and other enzymes discussed herein refers to any genetic modification in the microorganism in question which results in increased functionality of the enzymes and includes higher activity of the enzymes, reduced inhibition or degradation of the enzymes and overexpression of the enzymes. For example, gene copy number can be increased, expression levels can be increased by use of a promoter that gives higher levels of expression than that of the native promoter, or a gene can be altered by genetic engineering or classical mutagenesis to increase the activity of an enzyme. One of the key enzymes in the mevalonate-dependent isoprenoid biosynthetic pathway is HMG-CoA reductase which catalyzes the reduction of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA). This is the primary rate-limiting and first irreversible step in the pathway, and increasing HMG-CoA reductase activity leads to higher yields of squalene and ergosterol in a wild-type strain of S. cerevisiae, and farnesol in an erg9 strain. One mechanism by which the action of HMG-CoA reductase can be increased is by reducing inhibition of the enzyme, by either genetically modifying the enzyme or by modifying the system to remove the inhibitor. For instance, both sterol and non-sterol products of the isoprenoid pathway feedback inhibit this enzyme (see, e.g., Parks and Casey, Annu. Rev. Microbiol. 49:95-116 (1995). Alternatively or in addition, the gene(s) coding for HMG-CoA reductase can be altered by genetic engineering or classical mutagenesis techniques to decrease or prevent inhibition. Also, the action of HMG-CoA reductase can be increased by increasing the gene copy number, by increasing the level of expression of the HMG-CoA reductase gene(s), or by altering the HMG-CoA reductase gene(s) by genetic engineering or classical mutagenesis to increase the activity of the enzyme. See U.S. Pat. No. 5,460,949, the entire contents of which are incorporated herein by reference. For example, truncated HMG-CoA reductases have been produced in which the regulatory domain has been removed and the use of gene copy numbers up to about six also gives increased activity. Id. See also, Downing et al., Biochem. Biophys. Res. Commun., 94, 974-79 (1980) describing two yeast mutants having increased levels of HMG-CoA reductase. Two isozymes of HMGCoA reductase, encoded by the HMG1 and HMG2 genes, exist in S. cerevisiae. The activity of these two isozymes is regulated by several mechanisms including regulation of transcription, regulation of translation, and for Hmg2p, degradation of the enzyme in the endoplasmic reticulum (Hampton and Rine, 1994; Donald, et. al. 1997). In both Hmg1p and Hmg2p, the catalytic domain resides in the .sup.-COOH terminal portion of the enzyme, while the regulatory domain resides in the membrane spanning NH.sub.2 -terminal region. It has been shown that overexpression of just the catalytic domain of Hmg1p in S. cerevisiae increases carbon flow through the isoprenoid pathway, resulting in overproduction of squalene (Saunders, et. al. 1995; Donald, et. al., 1997). The present inventors have expressed the catalytic domain of the S. cerevisiae Hmg2p in strains having a normal (i.e., unblocked) isoprenoid pathway and observed a significant increase in the production of squalene. Furthermore, overexpression of the catalytic domain of Hmg2p resulted in increased farnesol production in an erg9 mutant, and increased farnesol and GG production in an erg9 mutant overexpressing GGPP synthase, grown in fermentors.

Detailed Description Text - DETX (20):

A further embodiment of the present invention is the use of a microorganism which has been genetically modified to increase the action of GGPP synthase. Genes coding for this enzyme from a variety of sources, including bacteria, fungi, plants, mammals, and archaebacteria, have been identified. See, Brinkhaus et al., Arch. Biochem. Biophys., 266, 607-612 (1988); Carattoli et al., J. Biol. Chem., 266, 5854-59 (1991); Chen et al., J. Biol. Chem., 268, 11002-11007 (1993); Dogbo et al., Biochim. Biophys. Acta, 920, 140-148 (1987); Jiang et al., J. Biol. Chem., 270, 21793-99 (1995); Kuntz al., Plant J., 2, 25-34 (1992); Laferriere, et al., Biochim. Biophys. Acta, 1077, 167-72 (1991); Math et al., Proc. Natl. Acad. Sci. USA, 89, 6761-64 (1992); Ohnuma et al., J. Biol. Chem., 269, 14792-97 (1994); Sagami et al., Arch. Biochem. Biophys., 297, 314-20 (1992); Sagami et al., J. Biol. Chem., 269, 20561-66 (1994); Sandmann et al., J. Photochem. Photobiol. B: Biol., 18, 245-51 (1993); Scolnik et al., Plant Physiol., 104, 1469-70 (1994); Tachibana et al., Biosci. Biotech. Biochem., 7, 1129-33 (1993); Tachibana et al., J. Biochem., 114, 389-92 (1993); Wiedemann et al., Arch. Biochem. Biophys., 306, 152-57 (1993). Some organisms have a bifunctional enzyme which also serves as an FPP synthase, so it is involved in the overall conversion of IPP and DMAPP to FPP to GGPP (see FIG. 1). Some enzymes, such as those found in plants, have relaxed specificity, converting IPP and DMAPP to GGPP (see FIG. 1). Genetic modifications of GGPP synthase, as used herein, encompass engineering a monofunctional GGPP synthase or a bifunctional FPP/GGPP synthase to enhance the GGPP synthase activity component of the enzyme. A preferred GGPP synthase gene is the BTS1 gene from S. cervisiae. The BTS1 gene and its isolation are described in Jiang et al., J. Biol. Chem., 270, 21793-99 (1995) and copending application Ser. No. 08/761,344, filed on Dec. 6, 1996, the complete disclosure of which incorporated herein by reference. However, GGPP synthases of other hosts can be used, and the use of the bifunctional GGPP synthases may be particularly advantageous in terms of channeling carbon flow through FPP to GGPP, thereby avoiding loss of FPP to competing reactions in the cell.

Detailed Description Text - DETX (36):

In yeast, FPP is a branch point intermediate leading to the biosynthesis of sterols, heme, dolichol, ubiquinone, GGPP and farnesylated proteins. In E. coli, FPP serves as the substrate for octaprenyl pyrophosphate synthase in the pathway leading to ubiquinone. In bacteria that synthesize carotenoids, such as Erwina uredovora, FPP is converted to GGPP by GGPP synthase in the first step leading to the carotenoids. To increase the production of farnesol or GG, it is desirable to inactivate genes encoding enzymes that use FPP or GGPP as substrate, or to reduce the activity of the enzymes themselves, either through mutation or the use of specific enzyme inhibitors (as was discussed above for squalene synthase). In S. cerevisiae, for example, it may be advantageous to inactivate the first step in the pathway from FPP to heme, in addition to inactivating ERG9. As discussed earlier, in E. coli, partial or complete inactivation of the octaprenyl pyrophosphate synthase could increase the availability of FPP for conversion of farnesol. Finally, in bacteria that produce carotenoids, such as Erwina uredovora, elimination of GGPP synthase can increase the level of FPP for conversion of farnesol, while inactivating or reducing the activity of phytoene synthase (the crtB gene product) can increase the level of GGPP available for conversion to GG.

Detailed Description Text - DETX (47):

Sources of assimilable carbon which can be used in a suitable fermentation medium include, but are not limited to, sugars and their polymers, including, dextrin, sucrose, maltose, lactose, glucose, fructose, mannose, sorbose, arabinose and xylose; fatty acids; organic acids such as acetate; primary

alcohols such as ethanol and n-propanol; and polyalcohols such as glycerine. Preferred carbon sources in the present invention include monosaccharides, disaccharides, and trisaccharides. The most preferred carbon source is glucose.

Detailed Description Text - DETX (93):

The mutants were assayed for squalene synthase activity. The squalene synthase assay involved incubation of a cell-free extract with FPP in the presence the reduced form of nicotinamide adenine dinucleotide phosphate, (NADPH), extraction into ethyl acetate, and squalene detection by GC. Specifically, 0.1 M Tris/HCl pH7 (X.mu.L), 0.1 M DTT (2 .mu.L), 0.1 M MgCl.sub.2 (10 .mu.L), 0.1 M NADPH (4 .mu.L), 1 mg/mL FPP (6 .mu.L), and cell-free extract (7000.times. g supernatant) (Y.mu.L), where X+Y=178 .mu.L to make 200 .mu.L total, were combined. This mixture was incubated in glass tubes at 37.degree. C. for 40 min. Then, it was extracted with 0.15 mL ethyl acetate. The extract was transferred to plastic vials and centrifuged at 15,000.times. g for 5 min. The ethyl acetate extract was analyzed using GC/MS.

Detailed Description Text - DETX (96):

For the FPP synthase assay, 0.1 M DTT (2 .mu.L), 0.1 M MgCl.sub.2 (2 .mu.L), 1 mg/mL IPP (6 .mu.L), 1 mg/mL GPP (6 .mu.L), cell-free extract (Y), and 0.1 M Tris/HCl (pH 7.0) (X .mu.L), where X+Y=84 .mu.L, were combined, and incubated at 37.degree. C. for 15 min. This mixture was then extracted twice with 0.3 mL hexane to remove pre-existing farnesol. Next, 0.1 mL of 2.times. glycine buffer (0.2 M glycine, 2 mM MgCl.sub.2, 2 mM Zn Cl.sub.2), pH 10.4, and 33 units of alkaline phosphatase were added, and the mixture was incubated at 37.degree. C. for an hour. The mixture was extracted with 0.1 mL of ethyl acetate and dried with sodium sulfate. Farnesol was determined with GC. A no-phosphatase control was included in each assay.

Detailed Description Text - DETX (168):

Approximately 5 .mu.g of purified erg9.DELTA.::HIS3 DNA was used to transform diploid strain CJ3A X CJ3B (a/.alpha., ura3/ura3, his3/his3, leu2/leu2, trp1/trp1, upc2/upc2, obtained from Dr. L. Parks, N.C. State University, Raleigh, N.C.) using the lithium acetate transformation procedure described in Gietz, et al. (1995). CJ3A X CJ3B is homozygous for the upc2 mutation. This mutation allows sterol uptake under aerobic conditions (Lewis, et al., 1988). It was believed that the upc2 mutation would allow the easy production of a haploid strain carrying a mutation in the erg9 gene. The histidine auxotrophy was necessary to select for strains carrying the plasmids that contain the functional HIS3 gene.

Detailed Description Text - DETX (170):

Hundreds of transformants were obtained. These HIS.sup.+ cells were then patched onto SC-His and allowed to grow for two more days. The transformants were then patched onto sporulation medium (Sherman, et. al, 1986). Sporulation medium contains (per liter of distilled water): 1% potassium acetate, 0.1% Bacto yeast extract, 0.05% dextrose and 2% Bacto agar. The patches were then allowed to grow and sporulate for 3-5 days. A portion of the cells was removed and placed in a solution of lyticase to digest the ascus cell wall. The cells were then spread in a thin line onto a YPD+2 mg/L ergosterol (YPDE) plate. The sporulated diploid cells form tetrads containing four spores, and the individual spores were separated using a micromanipulator. These individual haploid spores will germinate with each containing a single copy of the chromosomes.

Detailed Description Text - DETX (197):

HMG CoA reductase has been proposed to be the key enzyme regulating the flow of carbon through the isoprenoid pathway. In S. cerevisiae, two genes, HMG1 and HMG2, code for the two isozymes of HMG CoA reductase, designated HMG1p and HMG2p. Regulation of HMG CoA reductase is achieved through a combination of transcriptional and translational regulation as well as protein degradation. The segments of the HMG1 and HMG2 genes that encode the catalytic domains of the HMG1p and HMG2p isozymes have been cloned under transcriptional control of the strong promoter from the GPD (glyceraldehyde-3-phosphate dehydrogenase) gene. The plasmids containing these constructions (pRH127-3 and pRH124-31, containing the catalytic domains of HMG1p and HMG2p, respectively) were obtained from R. Hampton, U. C. San Diego. Strains of S. cerevisiae overexpressing the catalytic domain of HMG1p were reported to have an elevated flow of carbon through the isoprenoid pathway. This increased carbon flow was manifested as squalene and ergosterol overproduction (Donald, K. A. G., Hampton, R. Y., and Fritz, I. B., 1997, Effects of Overproduction of the Catalytic Domain of 3-Hydroxy-3-Methylglutaryl Coenzyme A Reductase on Squalene Synthesis in Saccharomyces cerevisiae. Applied and Environmental Microbiology 63:3341-3344).

Detailed Description Text - DETX (199):

In order to confirm that overexpressing HMG CoA reductase would result in increased carbon flow through the isoprenoid pathway in the strains derived from ATCC 28383, similar to other strains of S. cerevisiae (Donald, et. al., 1997), the strain SWY5, a ura3 mutant of the parental strain ATCC 28383, and SWE23-E9, the erg9 repaired version of EMS9-23 (strains described in Example 1.G. above), were transformed with plasmids pRH127-3 and pRH124-31. Transformations were performed using the LiOAc procedure (Gietz, et. al., 1995). Control strains carrying the empty vector YEp352 were also constructed. Representative transformants were tested for squalene and ergosterol production in a shake flask experiment. Cells were grown in 50 ml SCE-ura medium at 30.degree. C., with shaking at 180 rpm for 48 hours. At 24 hours, 10 ml of the culture was withdrawn, and the cells harvested for HMGCoA reductase assays as described in Example I.D. At the end of the 48-hour growth period, an aliquot of the cultures was used to inoculate flasks containing 50 ml YPD medium such that the starting OD.sub.600 of the cultures was 0.1. The cultures in YPD medium were grown for 72 hours at 30.degree. C., with shaking at 180 rpm, and then analyzed for dry cell weight, squalene and ergosterol accumulation. The extraction method used for this analysis was as follows. Ten ml of the culture was pelleted by centrifugation at 1,500.times. g for 10 minutes. The cell pellet was resuspended in 1 ml of deionized water, and the cells were repelleted. Cell pellets were resuspended in 1 ml of deionized water and disrupted by agitation with zirconium silicate beads (0.5 mm diameter). The broken cell suspension was saponified with 2.5 ml of a 0.2% solution of pyrogallol (in methanol) and 1.25 ml of 60% potassium hydroxide solution at 75 degrees C. for 1.5 hours. The samples were then extracted with 5 ml of hexane, vortexed for 3 minutes and centrifuged at 1,000.times. g for 20 minutes to separate the phases. The hexane layer was then analyzed by GC-MS as described in Example 1.B.

Detailed Description Text - DETX (200):

The data from this experiment are shown in Table 10. Compared to the control strains carrying only the Yep352 vector, the strains that overexpress the catalytic domains of either HMG1p or HMG2p contained high levels of HMGCoA reductase activity and elevated levels of squalene. However, neither of the

strains overexpressing HMG1p or HMG2p contained significantly increased levels of ergosterol. Nevertheless, these data show that increasing the activity of HMG CoA reductase in the MBNA1-13 strain lineage increases the carbon flow through the isoprenoid pathway.

Detailed Description Text - DETX (202):

Having shown that amplification of HMG1p or HMG2p increased carbon flow to squalene, whether overexpression of HMG1 or HMG2 would increase farnesol production in a strain carrying the erg9 mutation was tested. Strain SWE23-.DELTA.E91 (described in Example 1.G.) was transformed with plasmids pRHI27-3 or pRH124-31. Transformation of SWE23-.DELTA.E91 was accomplished using the LiOAc transformation procedure (Gietz et al, 1996). Approximately 2.5 .mu.g of pRH127-3 or pRH124-31 DNA were used in each transformation. Transformants were selected on SCE-ura plates, and several were chosen and restreaked for purification on SCE-ura plates. To test the effect of amplified HMG CoA reductase on farnesol production, representative transformants were grown for 48 hours in liquid SCE-ura medium. A control strain, SWE23-.DELTA.E91/YEp352, was also included in the experiment. At the end of the 48 hours growth period, aliquots of the cultures were used to inoculate flasks containing 50 ml of YPDE medium such that the initial OD.sub.600 was 0.5. These cultures were grown for 72 hours at 30.degree. C., with shaking at 180 rpm. At the end of the incubation period, samples were analyzed for dry cell weight and farnesol concentration. To confirm that the HMG CoA reductase genes were being overexpressed in the transformants, 20 ml of the cultures grown in SCE-ura were harvested by centrifugation at 1500.times. g for 10 minutes, and HMG CoA reductase activity measured using the permeabilized cell method described in Example I.D. The data from this experiment are shown in Table 11.

Detailed Description Text - DETX (220):

Strain EMS9-23 (ura3, erg9, described in Example 1.G.) was transformed with the plasmids described above using the LiOAc method, and transformants were selected on SCE-ura plates. Transformants were picked and restreaked on SCE-ura plates for purification. Representative transformants were tested for GG production. The strains were grown at 30.degree. C. for 48 hours in liquid SCE-ura medium, then used to inoculate flasks containing YPDE medium, such that the initial OD.sub.600 was 0.5. These cultures were incubated at 30.degree. C. with shaking for 72 hours and analyzed for dry cell weight, farnesol and GG levels. A second set of flasks containing 20 ml of SCE-ura medium was also inoculated from the same starting cultures and was grown for 48 hours. The cells from these flasks were harvested, washed with 10 ml 50 mM BisTris-Propane buffer, pH 7.0, and repelleted. The cell pellets were then used to prepare permeabilized cell suspensions for GGPP synthase assays. The cells were permeabilized by resuspending in 1 ml of 50 mM Bis-Tris-Propane buffer, pH 7.0, containing 0.1% Triton X100, and frozen at 80.degree. C. until needed. After thawing, the permeabilized cells were used for GGPP synthase assays. The GGPP synthase assay mixture contained 0.05M Bis-Tris propane buffer, pH 7.0 (X.mu.l), 0.1M dithiothreitol (1 .mu.l), 1 mg/ml FPP (5 .mu.l), 1 mg/ml IPP (5 .mu.l) and permeabilized cells (Y .mu.l), where X+Y=87 .mu.l. A control reaction was included in which the FPP and IPP were omitted. The assay mixtures were incubated at 37.degree. C. for 20 minutes. 0.1 ml of 2X glycine buffer (0.2M glycine, 2 mM MgCl.sub.2, 2 mM ZnCl.sub.2, pH 10.4) and 63 units of alkaline phosphatase (Sigma Chemical Co., St. Louis, Mo.) were added, and the mixtures were incubated for 60 minutes at 37.degree. C. The mixtures were then extracted with 0.2 ml 1:1 hexane:ethyl acetate, and analyzed for GG by GC/MS. GGPP synthase activity is expressed as nmol GGPP formed/min/mg protein.

Detailed Description Text - DETX (228):

To determine the effects of FPP synthase overexpression in erg9 mutant strains, representative transformants constructed as described above were grown in shake flask cultures and compared for growth, farnesol production, GG production and FPP synthase and GGPP synthase activities. The strains were grown with shaking at 30.degree. C. for 48 hours in liquid SCE-ura medium, then used to inoculate flasks containing YPDE medium, such that the initial OD.sub.600 was 0.5. These cultures were incubated at 30.degree. C. with shaking for an additional 72 hours and analyzed for dry cell weight, farnesol and GG levels. A second set of flasks containing 20 ml of SCE-ura medium was also inoculated from the same starting cultures and was grown for 48 hours. The cells from these flasks were harvested, washed with 10 ml 50 mM BisTris-Propane buffer, pH 7.0, and repelleted. The cell pellets were then used to prepare permeabilized cell suspensions for FPP and GGPP synthase assays. The cells were permeabilized by resuspending in 1 ml of 50 mM Bis-Tris-Propane buffer, pH 7.0, containing 0.1% Triton X100, and frozen at 80.degree. C. until needed. After thawing, the permeabilized cells were used for assays. The GGPP synthase assay mixture was the same as described in Example 5. The FPP synthase assay mixture contained 0.05 M Bis-Tris propane buffer, pH 7.0 (X .mu.l), 0.1M dithiothreitol (1 .mu.l), 0.5M MgCl.sub.2 (2 .mu.l), 1 mg/ml IPP (6 .mu.l), 1 mg/ml geranyldiphosphate (GPP) (6 .mu.l), and permeabilized cells (Y .mu.l), where X+Y=85 .mu.l. A control reaction was included in which the IPP and GPP were omitted. The assay mixtures were incubated at 37.degree. C. for 15 minutes. 0.1 ml of 2X glycine buffer (0.2M glycine, 2 mM MgCl.sub.2, 2 mM ZnCl.sub.2, pH 10.4) and 63 units of alkaline phosphatase (Sigma Chemical Co., St. Louis, Mo.) were added, and the mixtures were incubated for 60 minutes at 37.degree. C. The mixtures were then extracted with 0.2 ml 1:1 hexane:ethyl acetate, and analyzed for Farnesol by GC/MS. FPP synthase activity is expressed as nmol FPP formed/min/mg protein.

Detailed Description Text - DETX (258):

These data support the idea that strains with elevated levels of HMG CoA reductase produce more farnesol than a strain with normal levels of HMG CoA reductase. The data also indicate that a strain containing eight integrated copies of the HMG2cat gene produce essentially as much farnesol as a strain that carries more than 20 extrachromosomal copies of the HMG2cat gene as is the case with strain SW23B/pRH124-31. A strain containing a single integrated copy of the HMG2 cat gene produced more farnesol than the control strain, but slightly less than strains with more copies of the HMG2cat gene. In addition, the strains containing elevated HMG CoA reductase levels accumulated mevalonate in the culture while the strains with normal levels of HMG CoA reductase did not. This suggests that a step downstream of HMG CoA reductase limits carbon flux in the pathway once the HMG CoA reductase enzyme activity has been elevated (see Example 10). Carbon flux through the pathway is restricted by the activity of one of the enzymes downstream of HMG CoA reductase resulting in mevalonate accumulation in the medium.

Detailed Description Text - DETX (260):

This example shows the effects of over-expression of multiple isoprenoid pathway genes in a strain that has an erg9 mutation and elevated levels of HMG CoA reductase.

Detailed Description Text - DETX (261):

As shown in Examples 4 and 9, elevation of HMG CoA reductase levels led to higher carbon flux through the isoprenoid/sterol pathway. Over-expression of

other isoprenoid pathway genes in strains containing amplified HMG CoA reductase may further increase carbon flux through this pathway. Amplification of isoprenoid pathway genes in strains that have elevated levels of HMG CoA reductase as well as a defective erg9 gene may result in further elevation of farnesol levels. Also, amplification of isoprenoid pathway genes may result in further elevation of GG levels in strains that have a defective erg9 gene and have elevated levels of HMG CoA reductase and GGPP synthase.

Detailed Description Text - DETX (262):

To test these ideas, plasmids were constructed that allowed for the over-expression of multiple isoprenoid pathway genes. One of the plasmids provided for the over-expression of mevalonate kinase, phosphomevalonate kinase, and diphosphomevalonate decarboxylase, coded by the ERG12, ERG8, and ERG19 genes respectively. This plasmid is referred to as pSW77-69, and was constructed from DNA fragments obtained from a number of plasmids containing single isoprenoid pathway genes. The construction of those plasmids is described first.

Detailed Description Text - DETX (283):

These data show that a strain which over-expresses HMG CoA reductase accumulates the isoprenoid pathway intermediate mevalonate in the culture medium. Since mevalonate accumulation is not observed in strains with normal levels of HMG CoA reductase, this demonstrates that carbon flux through the isoprenoid pathway has been increased by HMG CoA reductase amplification to the point where another step subsequent to HMG CoA reductase limits the conversion of pathway intermediates. Furthermore, over-expression of the first three enzymes in the isoprenoid pathway, namely acetoacetyl CoA thiolase, HMG CoA synthase, and HMG CoA reductase (coded by ERG 13, ERG10, and HMG2cat, respectively) led to even higher accumulation of mevalonate in the medium. This demonstrates that carbon flux into the isoprenoid pathway has been increased further by amplification of the first three steps, and emphasizes that one of the enzymes downstream of HMG CoA reductase limits conversion of isoprenoid pathway intermediates. Since mevalonate serves as a precursor to farnesol and GG, the modifications described above can be used to increase carbon flux into the isoprenoid pathway, and, if the mevalonate can be more efficiently metabolized, can lead to increased farnesol and GG accumulation.

Detailed Description Text - DETX (293):

In order to construct strains of E. coli that accumulate elevated levels of farnesol, plasmids were constructed for the over-expression in E. coli of the genes listed above. In some cases, additional genes adjacent to the known isoprenoid pathway gene were included in the cloned DNA.

Detailed Description Text - DETX (294):

The idi gene coding for IPP isomerase was PCR amplified using the two oligonucleotides listed below and genomic DNA isolated from E. coli strain W3110. The oligonucleotides used to amplify the idi gene contained sequences corresponding to bases #42309 to #42335 and the reverse complement of #43699 to #43726 of Gene Bank Accession #U28375. The lower case letters are used for bases that were altered to create restriction endonuclease recognition sites, which are indicated in parentheses following the oligonucleotide sequence. A natural EcoRI site was used in VE145-5.

Detailed Description Text - DETX (302):

The resulting PCR product was digested with EcoRI and HindIII, and ligated into pET24d(+) (Novagen) so that expression of the ctrE gene was controlled by the T7 promoter. The resulting plasmid is referred to as pKL19-63. This plasmid can be transformed into E. coli strains such as BL21(DE3) (available from Novagen) which contains an IPTG inducible gene coding for T7 polymerase. This allows IPTG induction of the crtE gene in this strain. The T7 promoter/crtE gene fusion can be cut from pKL19-63 using BglII and HindIII, and ligated into BamHI, HindIII digested pACYC184 (Accession #X06403) to construct a plasmid relying on chloramphenicol resistance for selection. This plasmid contains the p15A origin of replication and would be compatible with plasmids containing the ColE1 origin of replication such as the clones carrying the E. coli isoprenoid pathway genes described above. These latter plasmids confer ampicillin resistance, and so E. coli transformants can be obtained that carry both the crtE plasmid and the plasmid containing the dxs, dxr, idi, and ispA genes by transforming E. coli with both plasmids and selecting for resistance to chloramphenicol and ampicillin. Therefore, strains of E. coli BL21(DE3) will be obtained that contain plasmids for over-expression deoxyxylulose-5-phosphate synthase, deoxyxylulose-5-phosphate reductoisomerase, IPP isomerase, FPP synthase, and GGPP synthase. These strains will be tested for production of GG in shake flask and fermentation experiments.

Detailed Description Paragraph Table - DETL (33):

TABLE 17 Solvent Farnesol (ng) Hexane 3273 Chloroform 2262 Ethyl acetate 3549 N-heptane 3560 Hexadecane Not determined* Dodecane 3529 Toluene 1055 Carbon tetrachloride 2023 Isobutyl Alcohol 2892 1-Octanol 1814

*Solvent peak coeluted with farnesol, making quantification of farnesol impossible using this method.

US-PAT-NO: 6660507

DOCUMENT-IDENTIFIER: US 6660507 B2

TITLE: Genes involved in isoprenoid compound production

DATE-ISSUED: December 9, 2003

INVENTOR-INFORMATION:

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APPL-NO: 09/ 934903

DATE FILED: August 22, 2001

PARENT-CASE:

This application claims the benefit of U.S. Provisional Application No. 60/229,907, filed Sep. 1, 2000.

US-CL-CURRENT: 435/166, 435/167 , 435/183 , 435/252.3 , 435/254.2 , 435/325 , 536/23.2

ABSTRACT:

Genes have been isolated from Methylomonas 16a sp. encoding the isoprenoid biosynthetic pathway. The genes and gene products are the first isolated from a Methylomonas strain that is capable of utilizing single carbon (C1) substrates as energy sources. The genes and gene products of the present invention may be used in a variety of ways for the production of isoprenoid compounds in a variety of organisms.

8 Claims, 5 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 4

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TITLE - TI (1):
Genes involved in isoprenoid compound production

Brief Summary Text - BSTX (6):
For many years, it was accepted that IPP was synthesized through the well-known acetate/mevalonate pathway. However, recent studies have

demonstrated that the mevalonate-dependent pathway does not operate in all living organisms. An alternate mevalonate-independent pathway for IPP biosynthesis was initially characterized in bacteria and later also in green algae and higher plants (Horbach et al., FEMS Microbiol. Lett. 111:135-140 (1993); Rohmer et al, Biochem. 295: 517-524 (1993); Schwender et al., Biochem. 316: 73-80 (1996); Eisenreich et al., Proc. Natl. Acad. Sci. USA 93: 6431-6436 (1996)).

Brief Summary Text - BSTX (16):

Further down in the isoprenoid biosynthesis pathway, more genes are involved in the synthesis of specific isoprenoids. As an example, the crtN gene was found in Heliobacillus mobilis (Xiang et al., Proc. Natl. Acad. Sci. USA 95:14851-14856 (1998)) to encode diapophytoene dehydrogenase is a part of the carotenoid biosynthesis pathway.

Brief Summary Text - BSTX (17):

Although most of the genes involved in the isoprenoid pathways are known, the genes involved in the isoprenoid pathway of methanotrophic bacteria are not described in the existing literature. However, there are many pigmented methylotrophic and methanotrophic bacteria, which suggests that the ability to produce carotenoid pigments is widespread in these bacteria and therefore the genes must be widespread in these bacteria. Applicants have isolated a number of unique open reading frames encoding enzymes of the isoprenoid biosynthesis pathway from a Methylomonas sp.

Brief Summary Text - BSTX (24):

Similarly the invention provides a method of regulating isoprenoid biosynthesis in an organism comprising, over-expressing at least one isoprenoid gene selected from the group consisting of SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17 and 23 in an organism such that the isoprenoid biosynthesis is altered in the organism.

Brief Summary Text - BSTX (25):

In another embodiment the invention provides a mutated gene encoding a isoprenoid enzyme having an altered biological activity produced by a method comprising the steps of (i) digesting a mixture of nucleotide sequences with restriction endonucleases wherein said mixture comprises: a) a native isoprenoid gene; b) a first population of nucleotide fragments which will hybridize to said native isoprenoid gene; c) a second population of nucleotide fragments which will not hybridize to said native isoprenoid gene;

Brief Summary Text - BSTX (26):

wherein a mixture of restriction fragments are produced; (ii) denaturing said mixture of restriction fragments; (iii) incubating the denatured said mixture of restriction fragments of step (ii) with a polymerase; (iv) repeating steps (ii) and (iii) wherein a mutated isoprenoid gene is produced encoding a protein having an altered biological activity.

Drawing Description Text - DRTX (3):

FIG. 2 shows two gene clusters contain genes in the isoprenoid pathway. One cluster contains the ispD, ispF and pyrG genes, and the other cluster contains the crtN1 and crtN2 genes.

Detailed Description Text - DETX (51):
The term "high growth methanotrophic bacterial strain" refers to a bacterium capable of growth with methane or methanol as the sole carbon and energy source and which possess a functional Embden-Meyerof carbon flux pathway resulting in a high rate of growth and yield of cell mass per gram of C1 substrate metabolized. The specific "high growth methanotrophic bacterial strain" described herein is referred to as "Methylomonas 16a" or "16a", which terms are used interchangeably.

Detailed Description Text - DETX (80):
A variety of nucleotide sequences have been isolated from Methylomonas 16a encoding gene products involved in the isoprenoid production pathway. ORF's 1-6 for example encode enzymes early in the isoprenoid pathway (FIG. 1) leading to IPP, which is the precursor of all isoprenoid compounds. ORF 7 encodes the IspA enzyme that is involved in elongation by condensing IPP precursors. ORF 8 and ORF 9 are involved more specifically in carotenoid production.

Detailed Description Text - DETX (105):
Of particular interest in the present invention are high growth obligate methanotrophs having an energetically favorable carbon flux pathway. For example Applicants have discovered a specific strain of methanotroph having several pathway features which make it particularly useful for carbon flux manipulation. This type of strain has served as the host in the present application and is known as Methylomonas 16a (ATCC PTA 2402).

Detailed Description Text - DETX (107):
A particularly novel and useful feature of the Embden-Meyerhof pathway in strain 16a is that the key phosphofructokinase step is pyrophosphate dependent instead of ATP dependent. This feature adds to the energy yield of the pathway by using pyrophosphate instead of ATP. Because of its significance in providing an energetic advantage to the strain, this gene in the carbon flux pathway is considered diagnostic for the present strain.

Detailed Description Text - DETX (117):
Knowledge of the sequence of the present genes will be useful in manipulating the isoprenoid biosynthetic pathways in any organism having such a pathway and particularly in methanotrophs. Methods of manipulating genetic pathways are common and well known in the art. Selected genes in a particularly pathway may be upregulated or down regulated by variety of methods. Additionally, competing pathways organism may be eliminated or sublimated by gene disruption and similar techniques.

Detailed Description Text - DETX (123):
Within the context of the present invention it may be useful to modulate the expression of the identified isoprenoid pathway by any one of the above described methods. For example, the present invention provides a number of genes encoding key enzymes in the terpenoid pathway leading to the production of pigments and smaller isoprenoid compounds. The isolated genes include the dxs and dsr genes, the ispA, D, E, F, and G genes, the pyrG gene and the crtN genes. In particular it may be useful to up-regulate the initial condensation of 3-carbon compounds (pyruvate and C1 aldehyde group, D-glyceraldehyde 3-Phosphate), to yield the 5-carbon compound D-1-deoxyxylulose-5-phosphate mediated by the dxs gene. Alternatively, if it is desired to produce a

specific non-pigmented isoprenoid, it may be desirable to disrupt various genes at the downstream end of the pathway. For example, the crtN gene is known to encode diapophytoene dehydrogenase, which is a part of the carotenoid biosynthesis pathway. It may be desirable to use gene disruption or antisense inhibition of this gene if a smaller, upstream terpenoid is the desired product of the pathway.

Detailed Description Text - DETX (146):

The activity of the present genes and gene products has been confirmed by studies showing the increase in carotenoid production in the source strain, Methylomonas 16a. By overexpressing genes that are early in the isoprenoid pathway, dxr and dxs, an increase in carotenoid production was observed in Methylomonas 16a cells. Briefly, genes dxr and dxs were overexpressed in Methylomonas 16a by cloning them into the low-copy, broad-host range plasmid pTJS75::lacZ:Tn5Kn (Schmidhauser and Helinsk, J. Bacteriology. Vol.164:446-455 (1985)). The method for cloning genes into the host plasmid is well known in the art. Genes were amplified from the Methylomonas 16a genome via PCR with the following primers.

Detailed Description Text - DETX (169):

200 to 500 .mu.g of chromosomal DNA was resuspended in a solution of 300 mM sodium acetate, 10 mM tris-HCl, 1 mM Na-EDTA, and 30% glycerol, and sheared at 12 psi for 60 sec in an Aeromist Downdraft Nebulizer chamber (IBI Medical products, Chicago, Ill.). The DNA was precipitated, resuspended and treated with Bal31 nuclease. After size fractionation, a fraction (2.0 kb, or 5.0 kb) was excised, cleaned and a two-step ligation procedure was used to produce a high titer library with greater than 99% single inserts.

US-PAT-NO: 6531303

DOCUMENT-IDENTIFIER: US 6531303 B1

TITLE: Method of producing geranylgeraniol

DATE-ISSUED: March 11, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
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APPL-NO: 09/ 350275

DATE FILED: July 6, 1999

PARENT-CASE:

REFERENCE TO RELATED APPLICATION

This application claims priority from U.S. Provisional Application Serial No. 60/091,964, filed Jul. 6, 1998, entitled "Production of Farnesol and Geranylgeraniol."

US-CL-CURRENT: 435/155, 435/193 , 435/254.21 , 435/320.1 , 435/441 , 435/471

ABSTRACT:

The invention provides a biological method of producing geranylgeraniol.

14 Claims, 24 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 24

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Detailed Description Text - DETX (8):

Suitable biological systems for producing farnesol and GG include prokaryotic and eukaryotic cell cultures and cell-free systems. Preferred biological systems include fungal, bacterial and microalgal systems. More preferred biological systems are fungal cell cultures, more preferably a yeast cell culture, and most preferably a Saccharomyces cerevisiae cell culture. Fungi are preferred since they have a long history of use in industrial processes and can be manipulated by both classical microbiological and genetic engineering techniques. Yeast, in particular, are well-characterized genetically. Indeed, the entire genome of S. cerevisiae has been sequenced, and the genes coding for enzymes in the isoprenoid pathway have already been cloned. Also, S. cerevisiae grows to high cell densities, and amounts of squalene and ergosterol (see FIG. 1) up to 16% of cell dry weight have been reported in genetically-engineered strains. For a recent review of the isoprenoid pathway in yeast, see Parks and Casey, Annu. Rev. Microbiol. 49:95-116 (1995).

Detailed Description Text - DETX (18):

A further embodiment of the present invention is the use of a microorganism which has been genetically modified to increase the action of HMG-CoA reductase. It should be noted that reference to increasing the action of HMG-CoA reductase and other enzymes discussed herein refers to any genetic modification in the microorganism in question which results in increased functionality of the enzymes and includes higher activity of the enzymes, reduced inhibition or degradation of the enzymes and overexpression of the enzymes. For example, gene copy number can be increased, expression levels can be increased by use of a promoter that gives higher levels of expression than that of the native promoter, or a gene can be altered by genetic engineering or classical mutagenesis to increase the activity of an enzyme. One of the key enzymes in the mevalonate-dependent isoprenoid biosynthetic pathway is HMG-CoA reductase which catalyzes the reduction of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA). This is the primary rate-limiting and first irreversible step in the pathway, and increasing HMG-CoA reductase activity leads to higher yields of squalene and ergosterol in a wild-type strain of S. cerevisiae, and farnesol in an erg9 strain. One mechanism by which the action of HMG-CoA reductase can be increased is by reducing inhibition of the enzyme, by either genetically modifying the enzyme or by modifying the system to remove the inhibitor. For instance, both sterol and non-sterol products of the isoprenoid pathway feedback inhibit this enzyme (see, e.g., Parks and Casey, Annu. Rev. Microbiol. 49:95-116 (1995). Alternatively or in addition, the gene(s) coding for HMG-CoA reductase can be altered by genetic engineering or classical mutagenesis techniques to decrease or prevent inhibition. Also, the action of HMG-CoA reductase can be increased by increasing the gene copy number, by increasing the level of expression of the HMG-CoA reductase gene(s), or by altering the HMG-CoA reductase gene(s) by genetic engineering or classical mutagenesis to increase the activity of the enzyme. See U.S. Pat. No. 5,460,949, the entire contents of which are incorporated herein by reference. For example, truncated HMG-CoA reductases have been produced in which the regulatory domain has been removed and the use of gene copy numbers up to about six also gives increased activity. Id. See also, Downing et al., Biochem. Biophys. Res. Commun., 94, 974-79 (1980) describing two yeast mutants having increased levels of HMG-CoA reductase. Two isozymes of HMGCoA reductase, encoded by the HMG1 and HMG2 genes, exist in S. cerevisiae. The activity of these two isozymes is regulated by several mechanisms including regulation of transcription, regulation of translation, and for Hmg2p, degradation of the enzyme in the endoplasmic reticulum (Hampton and Rine, 1994; Donald, et. al. 1997). In both Hmg1p and Hmg2p, the catalytic domain resides in the COOH terminal portion of the enzyme, while the regulatory domain resides in the membrane spanning NH.sub.2 -terminal region. It has been shown that overexpression of just the catalytic domain of Hmg1p in S. cerevisiae increases carbon flow through the isoprenoid pathway, resulting in overproduction of squalene (Saunders, et. al. 1995; Donald, et. al., 1997). The present inventors have expressed the catalytic domain of the S. cerevisiae Hmg2p in strains having a normal (i.e., unblocked) isoprenoid pathway and observed a significant increase in the production of squalene. Furthermore, overexpression of the catalytic domain of Hmg2p resulted in increased farnesol production in an erg9 mutant, and increased farnesol and GG production in an erg9 mutant overexpressing GGPP synthase, grown in fermentors.

Detailed Description Text - DETX (20):

A further embodiment of the present invention is the use of a microorganism which has been genetically modified to increase the action of GGPP synthase. Genes coding for this enzyme from a variety of sources, including bacteria,

fungi, plants, mammals, and archaebacteria, have been identified. See, Brinkhaus et al., Arch. Biochem. Biophys., 266, 607-612 (1988); Carattoli et al., J. Biol. Chem., 266, 5854-59 (1991); Chen et al., J. Biol. Chem., 268, 11002-11007 (1993); Dogbo et al., Biochim. Biophys. Acta, 920, 140-148 (1987); Jiang et al., J. Biol. Chem., 270, 21793-99 (1995); Kuntz al., Plant J., 2, 25-34 (1992); Laferriere, et al., Biochim. Biophys. Acta, 1077, 167-72 (1991); Math et al., Proc. Natl. Acad. Sci. USA, 89, 6761-64 (1992); Ohnuma et al., J. Biol. Chem., 269, 14792-97 (1994); Sagami et al., Arch. Biochem. Biophys., 297, 314-20 (1992); Sagami et al., J. Biol. Chem., 269, 20561-66 (1994); Sandmann et al., J. Photochem. Photobiol. B: Biol., 18, 245-51 (1993); Scolnik et al., Plant Physiol., 104, 1469-70 (1994); Tachibana et al., Biosci. Biotech. Biochem., 7, 1129-33 (1993); Tachibana et al., J. Biochem., 114, 389-92 (1993); Wiedemann et al., Arch. Biochem. Biophys., 306, 152-57 (1993). Some organisms have a bifunctional enzyme which also serves as an FPP synthase, so it is involved in the overall conversion of IPP and DMAPP to FPP to GGPP (see FIG. 1). Some enzymes, such as those found in plants, have relaxed specificity, converting IPP and DMAPP to GGPP (see FIG. 1). Genetic modifications of GGPP synthase, as used herein, encompass engineering a monofunctional GGPP synthase or a bifunctional FPP/GGPP synthase to enhance the GGPP synthase activity component of the enzyme. A preferred GGPP synthase gene is the BTS1 gene from S. cervisiae. The BTS1 gene and its isolation are described in Jiang et al., J. Biol. Chem., 270, 21793-99 (1995) and copending application Ser. No. 08/761,344, filed on Dec. 6, 1996, the complete disclosure of which incorporated herein by reference. However, GGPP synthases of other hosts can be used, and the use of the bifunctional GGPP synthases may be particularly advantageous in terms of channeling carbon flow through FPP to GGPP, thereby avoiding loss of FPP to competing reactions in the cell.

Detailed Description Text - DETX (33):

Blocking Pathways that Compete for FPP or GGPP. In yeast, FPP is a branch point intermediate leading to the biosynthesis of sterols, heme, dolichol, ubiquinone, GGPP and farnesylated proteins. In E. coli, FPP serves as the substrate for octaprenyl pyrophosphate synthase in the pathway leading to ubiquinone. In bacteria that synthesize carotenoids, such as Erwina uredovora, FPP is converted to GGPP by GGPP synthase in the first step leading to the carotenoids. To increase the production of farnesol or GG, it is desirable to inactivate genes encoding enzymes that use FPP or GGPP as substrate, or to reduce the activity of the enzymes themselves, either through mutation or the use of specific enzyme inhibitors (as was discussed above for squalene synthase). In S. cerevisiae, for example, it may be advantageous to inactivate the first step in the pathway from FPP to heme, in addition to inactivating ERG9. As discussed earlier, in E. coli, partial or complete inactivation of the octaprenyl pyrophosphate synthase could increase the availability of FPP for conversion of farnesol. Finally, in bacteria that produce carotenoids, such as Erwina uredovora, elimination of GGPP synthase can increase the level of FPP for conversion of farnesol, while inactivating or reducing the activity of phytoene synthase (the crtB gene product) can increase the level of GGPP available for conversion to GG.

Detailed Description Text - DETX (42):

Sources of assimilable carbon which can be used in a suitable fermentation medium include, but are not limited to, sugars and their polymers, including, dextrin, sucrose, maltose, lactose, glucose, fructose, mannose, sorbose, arabinose and xylose; fatty acids; organic acids such as acetate; primary alcohols such as ethanol and n-propanol; and polyalcohols such as glycerine. Preferred carbon sources in the present invention include monosaccharides, disaccharides, and trisaccharides. The most preferred carbon source is

glucose.

Detailed Description Text - DETX (88):
The mutants were assayed for squalene synthase activity. The squalene synthase assay involved incubation of a cell-free extract with FPP in the presence the reduced form of nicotinamide adenine dinucleotide phosphate, (NADPH), extraction into ethyl acetate, and squalene detection by GC. Specifically, 0.1 M Tris/HCl pH7 (X.mu.L), 0.1 M DTT (2 .mu.L), 0.1 M MgCl.sub.2 (10 .mu.L), 0.1 M NADPH (4 .mu.L), 1 mg/mL FPP (6 .mu.L), and cell-free extract (7000.times.g supernatant) (Y.mu.L), where X+Y=178 .mu.L to make 200 .mu.L total, were combined. This mixture was incubated in glass tubes at 37.degree. C. for 40 min. Then, it was extracted with 0.15 mL ethyl acetate. The extract was transferred to plastic vials and centrifuged at 15,000.times.g for 5 min. The ethyl acetate extract was analyzed using GC/MS.

Detailed Description Text - DETX (91):
For the FPP synthase assay, 0.1 M DTT (2 .mu.L), 0.1 M MgCl.sub.2 (2 .mu.L), 1 mg/mL IPP (6 .mu.L), 1 mg/mL GPP (6 .mu.L), cell-free extract (Y), and 0.1 M Tris/HCl (pH 7.0) (X .mu.L), where X+Y=84 .mu.L, were combined, and incubated at 37.degree. C. for 15 min. This mixture was then extracted twice with 0.3 mL hexane to remove pre-existing farnesol. Next, 0.1 mL of 2.times.glycine buffer (0.2 M glycine, 2 mM MgCl.sub.2, 2mM Zn Cl.sub.2) , pH 10.4, and 33 units of alkaline phosphatase were added, and the mixture was incubated at 37.degree. C. for an hour. The mixture was extracted with 0.1 mL of ethyl acetate and dried with sodium sulfate. Farnesol was determined with GC. A no-phosphatase control was included in each assay.

Detailed Description Text - DETX (151):
Approximately 5 .mu.g of purified erg9.DELTA.::HIS3 DNA was used to transform diploid strain CJ3A.times.CJ3B (a/.alpha., ura3/ura3, his3/his3, leu2/leu2, trp1/trp1, upc2/upc2, obtained from Dr. L. Parks, N.C. State University, Raleigh, N.C.) using the lithium acetate transformation procedure described in Gietz, et al. (1995). CJ3A.times.CJ3B is homozygous for the upc2 mutation. This mutation allows sterol uptake under aerobic conditions (Lewis, et al., 1988). It was believed that the upc2 mutation would allow the easy production of a haploid strain carrying a mutation in the erg9 gene. The histidine auxotrophy was necessary to select for strains carrying the plasmids that contain the functional HIS3 gene.

Detailed Description Text - DETX (153):
Hundreds of transformants were obtained. These HIS.sup.+ cells were then patched onto SC-His and allowed to grow for two more days. The transformants were then patched onto sporulation medium (Sherman, et. al, 1986). Sporulation medium contains (per liter of distilled water): 1% potassium acetate, 0.1% Bacto yeast extract, 0.05% dextrose and 2% Bacto agar. The patches were then allowed to grow and sporulate for 3-5 days. A portion of the cells was removed and placed in a solution of lyticase to digest the ascus cell wall. The cells were then spread in a thin line onto a YPD+2 mg/L ergosterol (YPDE) plate. The sporulated diploid cells form tetrads containing four spores, and the individual spores were separated using a micromanipulator. These individual haploid spores will germinate with each containing a single copy of the chromosomes.

Detailed Description Text - DETX (178):

HMG CoA reductase has been proposed to be the key enzyme regulating the flow of carbon through the isoprenoid pathway. In S. cerevisiae, two genes, HMG1 and HMG2, code for the two isozymes of HMG CoA reductase, designated HMGIp and HMG2p. Regulation of HMG CoA reductase is achieved through a combination of transcriptional and translational regulation as well as protein degradation. The segments of the HMG1 and HMG2 genes that encode the catalytic domains of the HMGIp and HMG2p isozymes have been cloned under transcriptional control of the strong promoter from the GPD (glyceraldehyde-3-phosphate dehydrogenase) gene. The plasmids containing these constructions (pRH127-3 and pRH124-31, containing the catalytic domains of HMG1p and HMG2p, respectively) were obtained from R. Hampton, U.C. San Diego. Strains of S. cerevisiae overexpressing the catalytic domain of HMGIp were reported to have an elevated flow of carbon through the isoprenoid pathway. This increased carbon flow was manifested as squalene and ergosterol overproduction (Donald, K. A. G., Hampton, R. Y., and Fritz, I. B., 1997, Effects of Overproduction of the Catalytic Domain of 3-Hydroxy-3-Methylglutaryl Coenzyme A Reductase on Squalene Synthesis in Saccharomyces cerevisiae. Applied and Environmental Microbiology 63:3341-3344).

Detailed Description Text - DETX (180):

In order to confirm that overexpressing HMG CoA reductase would result in increased carbon flow through the isoprenoid pathway in the strains derived from ATCC 28383, similar to other strains of S. cerevisiae (Donald, et. al., 1997), the strain SWY5, a ura3 mutant of the parental strain ATCC 28383, and SWE23-E9, the erg9 repaired version of EMS9-23 (strains described in Example 1.G. above), were transformed with plasmids pRH127-3 and pRH124-31. Transformations were performed using the LiOAc procedure (Gietz, et. al., 1995). Control strains carrying the empty vector YEp352 were also constructed. Representative transformants were tested for squalene and ergosterol production in a shake flask experiment. Cells were grown in 50 ml SCE-ura medium at 30.degree. C., with shaking at 180 rpm for 48 hours. At 24 hours, 10 ml of the culture was withdrawn, and the cells harvested for HMGCoA reductase assays as described in Example I.D. At the end of the 48-hour growth period, an aliquot of the cultures was used to inoculate flasks containing 50 ml YPD medium such that the starting OD.sub.600 of the cultures was 0.1. The cultures in YPD medium were grown for 72 hours at 30.degree. C., with shaking at 180 rpm, and then analyzed for dry cell weight, squalene and ergosterol accumulation. The extraction method used for this analysis was as follows. Ten ml of the culture was pelleted by centrifugation at 1,500.times.g for 10 minutes. The cell pellet was resuspended in 1 ml of deionized water, and the cells were repelleted. Cell pellets were resuspended in 1 ml of deionized water and disrupted by agitation with zirconium silicate beads (0.5 mm diameter). The broken cell suspension was saponified with 2.5 ml of a 0.2% solution of pyrogallol (in methanol) and 1.25 ml of 60% potassium hydroxide solution at 75 degrees C. for 1.5 hours. The samples were then extracted with 5 ml of hexane, vortexed for 3 minutes and centrifuged at 1,000.times.g for 20 minutes to separate the phases. The hexane layer was then analyzed by GC-MS as described in Example 1.B.

Detailed Description Text - DETX (181):

The data from this experiment are shown in Table 10. Compared to the control strains carrying only the Yep352 vector, the strains that overexpress the catalytic domains of either HMG1p or HMG2p contained high levels of HMGCoA reductase activity and elevated levels of squalene. However, neither of the strains overexpressing HMG1p or HMG2p contained significantly increased levels of ergosterol. Nevertheless, these data show that increasing the activity of HMG CoA reductase in the MBNA1-13 strain lineage increases the carbon flow

through the isoprenoid pathway.

Detailed Description Text - DETX (183):

Having shown that amplification of HMGlp or HMG2p increased carbon flow to squalene, whether overexpression of HMG1 or HMG2 would increase farnesol production in a strain carrying the erg9 mutation was tested. Strain SWE23-.DELTA.E91 (described in Example 1.G.) was transformed with plasmids pRHI27-3 or pRH124-31. Transformation of SWE23-.DELTA.E91 was accomplished using the LiOAc transformation procedure (Gietz et al, 1996). Approximately 2.5 .mu.g of pRH127-3 or pRH124-31 DNA were used in each transformation. Transformants were selected on SCE-ura plates, and several were chosen and restreaked for purification on SCE-ura plates. To test the effect of amplified HMG CoA reductase on farnesol production, representative transformants were grown for 48 hours in liquid SCE-ura medium. A control strain, SWE23-.DELTA.E91/YEp352, was also included in the experiment. At the end of the 48 hours growth period, aliquots of the cultures were used to inoculate flasks containing 50 ml of YPDE medium such that the initial OD.sub.600 was 0.5. These cultures were grown for 72 hours at 30.degree. C., with shaking at 180 rpm. At the end of the incubation period, samples were analyzed for dry cell weight and farnesol concentration. To confirm that the HMG CoA reductase genes were being overexpressed in the transformants, 20 ml of the cultures grown in SCE-ura were harvested by centrifugation at 1500.times.g for 10 minutes, and HMG CoA reductase activity measured using the permeabilized cell method described in Example I.D. The data from this experiment are shown in Table 11.

Detailed Description Text - DETX (201):

Strain EMS9-23 (ura3, erg9, described in Example 1.G.) was transformed with the plasmids described above using the LiOAc method, and transformants were selected on SCE-ura plates. Transformants were picked and restreaked on SCE-ura plates for purification. Representative transformants were tested for GG production. The strains were grown at 30.degree. C. for 48 hours in liquid SCE-ura medium, then used to inoculate flasks containing YPDE medium, such that the initial OD.sub.600 was 0.5. These cultures were incubated at 30.degree. C. with shaking for 72 hours and analyzed for dry cell weight, farnesol and GG levels. A second set of flasks containing 20 ml of SCE-ura medium was also inoculated from the same starting cultures and was grown for 48 hours. The cells from these flasks were harvested, washed with 10 ml 50 mM BisTris-Propane buffer, pH 7.0, and repelleted. The cell pellets were then used to prepare permeabilized cell suspensions for GGPP synthase assays. The cells were permeabilized by resuspending in 1 ml of 50 mM Bis-Tris-Propane buffer, pH 7.0, containing 0.1% Triton X100, and frozen at 80.degree. C. until needed. After thawing, the permeabilized cells were used for GGPP synthase assays. The GGPP synthase assay mixture contained 0.05M Bis-Tris propane buffer, pH 7.0 (X.mu.l), 0.1M dithiothreitol (1 .mu.l), 1 mg/ml FPP (5 .mu.l), 1 mg/ml IPP (5 .mu.l) and permeabilized cells (Y .mu.l), where X+Y=87 .mu.l. A control reaction was included in which the FPP and IPP were omitted. The assay mixtures were incubated at 37.degree. C. for 20 minutes. 0.1 ml of 2.times. glycine buffer (0.2M glycine, 2 mM MgCl.sub.2, 2 mM ZnCl.sub.2, pH 10.4) and 63 units of alkaline phosphatase (Sigma Chemical Co., St. Louis, Mo.) were added, and the mixtures were incubated for 60 minutes at 37.degree. C. The mixtures were then extracted with 0.2 ml 1:1 hexane:ethyl acetate, and analyzed for GG by GC/MS. GGPP synthase activity is expressed as nmol GGPP formed/min/mg protein.

Detailed Description Text - DETX (209):

To determine the effects of FPP synthase overexpression in erg9 mutant strains, representative transformants constructed as described above were grown in shake flask cultures and compared for growth, farnesol production, GG production and FPP synthase and GGPP synthase activities. The strains were grown with shaking at 30.degree. C. for 48 hours in liquid SCE-ura medium, then used to inoculate flasks containing YPDE medium, such that the initial OD.sub.600 was 0.5. These cultures were incubated at 30.degree. C. with shaking for an additional 72 hours and analyzed for dry cell weight, farnesol and GG levels. A second set of flasks containing 20 ml of SCE-ura medium was also inoculated from the same starting cultures and was grown for 48 hours. The cells from these flasks were harvested, washed with 10 ml 50 mM BisTris-Propane buffer, pH 7.0, and repelleted. The cell pellets were then used to prepare permeabilized cell suspensions for FPP and GGPP synthase assays. The cells were permeabilized by resuspending in 1 ml of 50 mM Bis-Tris-Propane buffer, pH 7.0, containing 0.1% Triton X100, and frozen at 80.degree. C. until needed. After thawing, the permeabilized cells were used for assays. The GGPP synthase assay mixture was the same as described in Example 5. The FPP synthase assay mixture contained 0.05 M Bis-Tris propane buffer, pH 7.0 (X .mu.l), 0.1M dithiothreitol (1 .mu.l), 0.5M MgCl.sub.2 (2 .mu.l), 1 mg/ml IPP (6 .mu.l), 1 mg/ml geranyldiphosphate (GPP) (6 .mu.l), and permeabilized cells (Y .mu.l), where X+Y=85 .mu.l. A control reaction was included in which the IPP and GPP were omitted. The assay mixtures were incubated at 37.degree. C. for 15 minutes. 0.1 ml of 2.times. glycine buffer (0.2M glycine, 2 mM MgCl.sub.2, 2 mM ZnCl.sub.2, pH 10.4) and 63 units of alkaline phosphatase (Sigma Chemical Co., St. Louis, Mo.) were added, and the mixtures were incubated for 60 minutes at 37.degree. C. The mixtures were then extracted with 0.2 ml 1:1 hexane:ethyl acetate, and analyzed for Farnesol by GC/MS. FPP synthase activity is expressed as nmol FPP formed/min/mg protein.

Detailed Description Text - DETX (239):

These data support the idea that strains with elevated levels of HMG CoA reductase produce more farnesol than a strain with normal levels of HMG CoA reductase. The data also indicate that a strain containing eight integrated copies of the HMG2cat gene produce essentially as much farnesol as a strain that carries more than 20 extrachromosomal copies of the HMG2cat gene as is the case with strain SW23B/pRH124-31. A strain containing a single integrated copy of the HMG2 cat gene produced more farnesol than the control strain, but slightly less than strains with more copies of the HMG2cat gene. In addition, the strains containing elevated HMG CoA reductase levels accumulated mevalonate in the culture while the strains with normal levels of HMG CoA reductase did not. This suggests that a step downstream of HMG CoA reductase limits carbon flux in the pathway once the HMG CoA reductase enzyme activity has been elevated (see Example 10). Carbon flux through the pathway is restricted by the activity of one of the enzymes downstream of HMG CoA reductase resulting in mevalonate accumulation in the medium.

Detailed Description Text - DETX (241):

This example shows the effects of over-expression of multiple isoprenoid pathway genes in a strain that has an erg9 mutation and elevated levels of HMG CoA reductase.

Detailed Description Text - DETX (242):

As shown in Examples 4 and 9, elevation of HMG CoA reductase levels led to higher carbon flux through the isoprenoid/sterol pathway. Over-expression of other isoprenoid pathway genes in strains containing amplified HMG CoA reductase may further increase carbon flux through this pathway. Amplification

of isoprenoid pathway genes in strains that have elevated levels of HMG CoA reductase as well as a defective erg9 gene may result in further elevation of farnesol levels. Also, amplification of isoprenoid pathway genes may result in further elevation of GG levels in strains that have a defective erg9 gene and have elevated levels of HMG CoA reductase and GGPP synthase.

Detailed Description Text - DETX (243):

To test these ideas, plasmids were constructed that allowed for the over-expression of multiple isoprenoid pathway genes. One of the plasmids provided for the over-expression of mevalonate kinase, phosphomevalonate kinase, and diphosphomevalonate decarboxylase, coded by the ERG12, ERG8, and ERG19 genes respectively. This plasmid is referred to as pSW77-69, and was constructed from DNA fragments obtained from a number of plasmids containing single isoprenoid pathway genes. The construction of those plasmids is described first.

Detailed Description Text - DETX (264):

These data show that a strain which over-expresses HMG CoA reductase accumulates the isoprenoid pathway intermediate mevalonate in the culture medium. Since mevalonate accumulation is not observed in strains with normal levels of HMG CoA reductase, this demonstrates that carbon flux through the isoprenoid pathway has been increased by HMG CoA reductase amplification to the point where another step subsequent to HMG CoA reductase limits the conversion of pathway intermediates. Furthermore, over-expression of the first three enzymes in the isoprenoid pathway, namely acetoacetyl CoA thiolase, HMG CoA synthase, and HMG CoA reductase (coded by ERG 13, ERG10, and HMG2cat, respectively) led to even higher accumulation of mevalonate in the medium. This demonstrates that carbon flux into the isoprenoid pathway has been increased further by amplification of the first three steps, and emphasizes that one of the enzymes downstream of HMG CoA reductase limits conversion of isoprenoid pathway intermediates. Since mevalonate serves as a precursor to farnesol and GG, the modifications described above can be used to increase carbon flux into the isoprenoid pathway, and, if the mevalonate can be more efficiently metabolized, can lead to increased farnesol and GG accumulation.

Detailed Description Text - DETX (274):

In order to construct strains of E. coli that accumulate elevated levels of farnesol, plasmids were constructed for the over-expression in E. coli of the genes listed above. In some cases, additional genes adjacent to the known isoprenoid pathway gene were included in the cloned DNA.

Detailed Description Text - DETX (275):

The idi gene coding for IPP isomerase was PCR amplified using the two oligonucleotides listed below and genomic DNA isolated from E. coli strain W3110. The oligonucleotides used to amplify the idi gene contained sequences corresponding to bases #42309 to #42335 and the reverse complement of #43699 to #43726 of Gene Bank Accession #U28375. The lower case letters are used for bases that were altered to create restriction endonuclease recognition sites, which are indicated in parentheses following the oligonucleotide sequence. A natural EcoRI site was used in VE145-5. SEQ ID NO:42: VE145-5 5'-GGGATTCGAATTCTGTCGCGCTGTAAC-3' (EcoRI) SEQ ID NO:43: VE146-3 5'-TGggATCcGTAACGGCTTTAGCGAGCTG-3' (BamHI)

Detailed Description Text - DETX (283):

The resulting PCR product was digested with EcoRI and HindIII, and ligated into pET24d(+) (Novagen) so that expression of the ctrE gene was controlled by the T7 promoter. The resulting plasmid is referred to as pKL19-63. This plasmid can be transformed into E. coli strains such as BL21(DE3) (available from Novagen) which contains an IPTG inducible gene coding for T7 polymerase. This allows IPTG induction of the crtE gene in this strain. The T7 promoter/crtE gene fusion can be cut from pKL19-63 using BglII and HindIII, and ligated into BamHI, HindIII digested pACYC184 (Accession #X06403) to construct a plasmid relying on chloramphenicol resistance for selection. This plasmid contains the p15A origin of replication and would be compatible with plasmids containing the ColE1 origin of replication such as the clones carrying the E. coli isoprenoid pathway genes described above. These latter plasmids confer ampicillin resistance, and so E. coli transformants can be obtained that carry both the crtE plasmid and the plasmid containing the dxs, dxr, idi, and ispA genes by transforming E. coli with both plasmids and selecting for resistance to chloramphenicol and ampicillin. Therefore, strains of E. coli BL21(DE3) will be obtained that contain plasmids for over-expression deoxyxylulose-5-phosphate synthase, deoxyxylulose-5-phosphate reductoisomerase, IPP isomerase, FPP synthase, and GGPP synthase. These strains will be tested for production of GG in shake flask and fermentation experiments.

Detailed Description Paragraph Table - DETL (26):

TABLE 17 Solvent Farnesol (ng) Hexane 3273 Chloroform 2262 Ethyl acetate 3549 N-heptane 3560 Hexadecane Not determined* Dodecane 3529 Toluene 1055 Carbon tetrachloride 2023 Isobutyl Alcohol 2892 1-Octanol 1814

*Solvent peak coeluted with farnesol, making quantification of farnesol impossible using this method.

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TITLE: Method of vitamin production

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INVENTOR-INFORMATION:

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PARENT-CASE:

REFERENCE TO RELATED APPLICATION

This application claims priority from U.S. Provisional Application Ser. No. 60/091,868, filed Jul. 6, 1998, entitled "Method of Vitamin Production," and U.S. Provisional Application Ser. No. 60/091,983, filed Jul. 6, 1998, entitled "Method of Vitamin Production."

US-CL-CURRENT: 549/408, 435/132 , 435/170 , 435/171 , 549/413

ABSTRACT:

The invention provides a method of producing .alpha.-tocopherol and .alpha.-tocopheryl esters. The method comprises using a biological system to produce farnesol or geranylgeraniol. Then, the farnesol or geranylgernaiol is chemically converted into .alpha.-tocopherol or an .alpha.-tocopheryl ester.

20 Claims, 17 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 17

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Detailed Description Text - DETX (9):

Suitable biological systems for producing farnesol and GG include prokaryotic and eukaryotic cell cultures and cell-free systems. Preferred biological systems include fungal, bacterial and microalgal systems. More preferred biological systems are fungal cell cultures, more preferably a yeast cell culture, and most preferably a Saccharomyces cerevisiae cell culture. Fungi are preferred since they have a long history of use in industrial processes and can be manipulated by both classical microbiological and genetic engineering techniques. Yeast, in particular, are well-characterized genetically. Indeed, the entire genome of S. cerevisiae has been sequenced, and the genes coding for enzymes in the isoprenoid pathway have already been

cloned. Also, S. cerevisiae grows to high cell densities, and amounts of squalene and ergosterol (see FIG. 1) up to 16% of cell dry weight have been reported in genetically-engineered strains. For a recent review of the isoprenoid pathway in yeast, see Parks and Casey, Annu. Rev. Microbiol. 49:95-116 (1995).

Detailed Description Text - DETX (19):

A further embodiment of the present invention is the use of a microorganism which has been genetically modified to increase the action of HMG-CoA reductase. It should be noted that reference to increasing the action of HMG-CoA reductase and other enzymes discussed herein refers to any genetic modification in the microorganism in question which results in increased functionality of the enzymes and includes higher activity of the enzymes, reduced inhibition or degradation of the enzymes and overexpression of the enzymes. For example, gene copy number can be increased, expression levels can be increased by use of a promoter that gives higher levels of expression than that of the native promoter, or a gene can be altered by genetic engineering or classical mutagenesis to increase the activity of an enzyme. One of the key enzymes in the mevalonate-dependent isoprenoid biosynthetic pathway is HMG-CoA reductase which catalyzes the reduction of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA). This is the primary rate-limiting and first irreversible step in the pathway, and increasing HMG-CoA reductase activity leads to higher yields of squalene and ergosterol in a wild-type strain of S. cerevisiae, and farnesol in an erg9 strain. One mechanism by which the action of HMG-CoA reductase can be increased is by reducing inhibition of the enzyme, by either genetically modifying the enzyme or by modifying the system to remove the inhibitor. For instance, both sterol and non-sterol products of the isoprenoid pathway feedback inhibit this enzyme (see, e.g., Parks and Casey, Annu. Rev. Microbiol. 49:95-116 (1995). Alternatively or in addition, the gene(s) coding for HMG-CoA reductase can be altered by genetic engineering or classical mutagenesis techniques to decrease or prevent inhibition. Also, the action of HMG-CoA reductase can be increased by increasing the gene copy number, by increasing the level of expression of the HMG-CoA reductase gene(s), or by altering the HMG-CoA reductase gene(s) by genetic engineering or classical mutagenesis to increase the activity of the enzyme. See U.S. Pat. No. 5,460,949, the entire contents of which are incorporated herein by reference. For example, truncated HMG-CoA reductases have been produced in which the regulatory domain has been removed and the use of gene copy numbers up to about six also gives increased activity. Id. See also, Downing et al., Biochem. Biophys. Res. Commun., 94, 974-79 (1980) describing two yeast mutants having increased levels of HMG-CoA reductase. Two isozymes of HMGCoA reductase, encoded by the HMG1 and HMG2 genes, exist in S. cerevisiae. The activity of these two isozymes is regulated by several mechanisms including regulation of transcription, regulation of translation, and for Hmg2p, degradation of the enzyme in the endoplasmic reticulum (Hampton and Rine, 1994; Donald, et. al. 1997). In both Hmg1p and Hmg2p, the catalytic domain resides in the COOH terminal portion of the enzyme, while the regulatory domain resides in the membrane spanning NH.sub.2 -terminal region. It has been shown that overexpression of just the catalytic domain of Hmg1p in S. cerevisiae increases carbon flow through the isoprenoid pathway, resulting in overproduction of squalene (Saunders, et. al. 1995; Donald, et. al., 1997). The present inventors have expressed the catalytic domain of the S. cerevisiae Hmg2p in strains having a normal (i.e., unblocked) isoprenoid pathway and observed a significant increase in the production of squalene. Furthermore, overexpression of the catalytic domain of Hmg2p resulted in increased farnesol production in an erg9 mutant, and increased farnesol and GG production in an erg9 mutant overexpressing GGPP synthase, grown in fermentors.

Detailed Description Text - DETX (21):

A further embodiment of the present invention is the use of a microorganism which has been genetically modified to increase the action of GGPP synthase. Genes coding for this enzyme from a variety of sources, including bacteria, fungi, plants, mammals, and archaebacteria, have been identified. See, Brinkhaus et al., Arch. Biochem. Biophys., 266, 607-612 (1988); Carattoli et al., J. Biol. Chem., 266, 5854-59 (1991); Chen et al., J. Biol. Chem., 268, 11002-11007 (1993); Dogbo et al., Biochim. Biophys. Acta, 920, 140-148 (1987); Jiang et al., J. Biol. Chem., 270, 21793-99 (1995); Kuntz al., Plant J., 2, 25-34 (1992); Laferriere, et al., Biochim. Biophys. Acta, 1077, 167-72 (1991); Math et al., Proc. Natl. Acad. Sci. USA, 89, 6761-64 (1992); Ohnuma et al., J. Biol. Chem., 269, 14792-97 (1994); Sagami et al., Arch. Biochem. Biophys., 297, 314-20 (1992); Sagami et al., J. Biol. Chem., 269, 20561-66 (1994); Sandmann et al., J. Photochem. Photobiol. B: Biol., 18, 245-51 (1993); Scolnik et al., Plant Physiol., 104, 1469-70 (1994); Tachibana et al., Biosci. Biotech. Biochem., 7, 1129-33 (1993); Tachibana et al., J. Biochem., 114, 389-92 (1993); Wiedemann et al., Arch. Biochem. Biophys., 306, 152-57 (1993). Some organisms have a bifunctional enzyme which also serves as an FPP synthase, so it is involved in the overall conversion of IPP and DMAPP to FPP to GGPP (see FIG. 1). Some enzymes, such as those found in plants, have relaxed specificity, converting IPP and DMAPP to GGPP (see FIG. 1). Genetic modifications of GGPP synthase, as used herein, encompass engineering a bifunctional FPP/GGPP synthase to enhance the GGPP synthase activity component of the enzyme. A preferred GGPP synthase gene is the BTS1 gene from S. cervisiae. The BTS1 gene and its isolation are described in Jiang et al., J. Biol. Chem., 270, 21793-99 (1995) and copending application Ser. No. 08/761,344, filed on Dec. 6, 1996, the complete disclosure of which incorporated herein by reference. However, GGPP synthases of other hosts can be used, and the use of the bifunctional GGPP synthases may be particularly advantageous in terms of channeling carbon flow through FPP to GGPP, thereby avoiding loss of FPP to competing reactions in the cell.

Detailed Description Text - DETX (36):

In yeast, FPP is a branch point intermediate leading to the biosynthesis of sterols, heme, dolichol, ubiquinone, GGPP and farnesylated proteins. In E. coli, FPP serves as the substrate for octaprenyl pyrophosphate synthase in the pathway leading to ubiquinone. In bacteria that synthesize carotenoids, such as Erwina uredovora, FPP is converted to GGPP by GGPP synthase in the first step leading to the carotenoids. To increase the production of farnesol or GG, it is desirable to inactivate genes encoding enzymes that use FPP or GGPP as substrate, or to reduce the activity of the enzymes themselves, either through mutation or the use of specific enzyme inhibitors (as was discussed above for squalene synthase). In S. cerevisiae, for example, it may be advantageous to inactivate the first step in the pathway from FPP to heme, in addition to inactivating ERG9. As discussed earlier, in E. coli, partial or complete inactivation of the octaprenyl pyrophosphate synthase could increase the availability of FPP for conversion of farnesol. Finally, in bacteria that produce carotenoids, such as Erwina uredovora, elimination of GGPP synthase can increase the level of FPP for conversion of farnesol, while inactivating or reducing the activity of phytoene synthase (the crtB gene product) can increase the level of GGPP available for conversion to GG.

Detailed Description Text - DETX (47):

Sources of assimilable carbon which can be used in a suitable fermentation medium include, but are not limited to, sugars and their polymers, including, dextrin, sucrose, maltose, lactose, glucose, fructose, mannose, sorbose,

arabinose and xylose; fatty acids; organic acids such as acetate; primary alcohols such as ethanol and n-propanol; and polyalcohols such as glycerine. Preferred carbon sources in the present invention include monosaccharides, disaccharides, and trisaccharides. The most preferred carbon source is glucose.

Detailed Description Text - DETX (82):

FIG. 2 illustrates a particularly preferred embodiment of vitamin E synthesis from geranylgeraniol. The hydroxy group of geranylgeraniol 2-1 is protected as isobutyrate ester or other bulky ester using the corresponding acid or its derivative such as anhydride or acyl chloride. Geranylgeranylisobutyrate 2-2 is then selectively hydrogenated using a metal catalyst such as palladium on carbon to produce phytylisobutyrate 2-3. Hydrogenation of geranylgeranylisobutyrate 2-2 reduces the second, third and fourth olefin moieties without affecting the first olefin moiety. It is believed that the steric bulk of isobutyrate decreases the rate of hydrogenation of the first olefin moiety in protected geranylgeraniol, thus leading to a selective hydrogenation of olefin moieties. The hydroxy protecting group is then removed by hydrolysis under a basic condition to provide phytol 2-4. Reacting phytol 2-4 with 2,3,5-trimethyl hydroquinone 2-5 in the presence of an acid then results in a formation of vitamin E 2-6. Alternatively, phytylisobutyrate 2-3 can be deprotected and reacted with 2,3,5-trimethyl hydroquinone 2-5 in a single step by an acid catalyst to form vitamin E 2-6. Vitamin E 2-6 can further be transformed to a protected vitamin E such as vitamin E acetate 2-7 by acetylation of the free hydroxy moiety.

Detailed Description Text - DETX (84):

The step of selectively oxidizing the 2,3-carbon-carbon double bond in geranylgeraniol to produce geranylgeraniol-2,3-epoxide is carried out with any reagent known to selectively epoxidize the olefin functionality of allylic alcohols, such as combinations of tert-butyl hydroperoxide and vanadium or molybdenum catalysts as taught by the method of Sharpless and Michaelson, J. Am. Chem. Soc. 95, 6136 (1973). Hydrogen peroxide or other alkyl hydroperoxides could replace tert-butyl hydroperoxide, and inert solvents such as aromatic hydrocarbons, aliphatic hydrocarbons, cycloaliphatic hydrocarbons or aliphatic esters may be used. The term "aliphatic hydrocarbon" is used to include straight or branched chain alkanes having five to eight carbon atoms. Heptane is the most preferred aliphatic hydrocarbon. The term "cycloaliphatic hydrocarbon" is used to include C.sub.5 -C.sub.8 cycloalkanes and these substituted with one or more C.sub.1 -C.sub.4 alkyl groups. The term "aromatic hydrocarbon" is used to include benzene and benzene substituted with one to three C.sub.1 -C.sub.4 alkyl groups. Toluene is the most preferred aromatic hydrocarbon. The term "aliphatic esters" is used to include aliphatic esters having three to nine carbon having the formula R.sub.1 CO.sub.2 R.sub.2 wherein R.sub.1 and R.sub.2 independently represent a straight or branched chain C.sub.1 -C.sub.4 alkyl group, with ethyl acetate being the preferred aliphatic ester.

Detailed Description Text - DETX (88):

Specific exemplary compounds of generic terms used herein which are suitable for use in the present invention are as follows. Typical aromatic solvents include benzene, toluene, and o, m or p-xylene or mixtures thereof. Toluene is the preferred aromatic solvent. Typical aliphatic solvents include n-pentane, n-hexane, n-heptane and n-octane and isomers thereof, n-Heptane is the preferred aliphatic hydrocarbon. Typical cycloaliphatic hydrocarbons include cyclopentane, cyclohexane, methylcyclohexane and cycloheptane. Typical

aliphatic esters include methyl acetate, ethyl acetate, n-propyl acetate, n-butyl acetate, isopropyl acetate, methyl propionate, ethyl propionate, methyl butyrate and ethyl butyrate. Ethyl acetate is the preferred aliphatic ester. Typical alkanols and substituted alkanols include methanol, ethanol, n-propanol, isopropanol, n-butanol, isobutanol, 2-methoxyethanol, 2-ethoxyethanol and 2-isopropoxyethanol. Typical ethers include diethyl ether, diisopropyl ether, dibutyl ether, n-butyl methyl ether, t-butyl methyl ether and tetrahydrofuran. Typical useful Lewis acids include zinc chloride, aluminum chloride, boron trifluoride, ferric chloride and phosphoric acid. Zinc chloride is the preferred Lewis acid.

Detailed Description Text - DETX (123):

The mutants were assayed for squalene synthase activity. The squalene synthase assay involved incubation of a cell-free extract with FPP in the presence the reduced form of nicotinamide adenine dinucleotide phosphate, (NADPH), extraction into ethyl acetate, and squalene detection by GC. Specifically, 0.1 M Tris/HCl pH7 (X.mu.L), 0.1 M DTT (2 .mu.L), 0.1 M MgCl.sub.2 (10 .mu.L), 0.1 M NADPH (4 .mu.L), 1 mg/mL FPP (6 .mu.L), and cell-free extract (7000.times.g supernatant) (Y .mu.L), where X+Y=178 .mu.L to make 200 .mu.L total, were combined. This mixture was incubated in glass tubes at 37.degree. C. for 40 min. Then, it was extracted with 0.15 mL ethyl acetate. The extract was transferred to plastic vials and centrifuged at 15,000.times.g for 5 min. The ethyl acetate extract was analyzed using GC/MS.

Detailed Description Text - DETX (126):

For the FPP synthase assay, 0.1 M DTT (2 .mu.L), 0.1 M MgCl.sub.2 (2 .mu.L), 1 mg/mL IPP (6 .mu.L), 1 mg/mL GPP (6 .mu.L), cell-free extract (Y), and 0.1 M Tris/HCl (pH 7.0) (X .mu.L), where X+Y=84 .mu.L, were combined, and incubated at 37.degree. C. for 15 min. This mixture was then extracted twice with 0.3 mL hexane to remove pre-existing farnesol. Next, 0.1 mL of 2.times.glycine buffer (0.2 M glycine, 2 mM MgCl.sub.2, 2 mM Zn Cl.sub.2), pH 10.4, and 33 units of alkaline phosphatase were added, and the mixture was incubated at 37.degree. C. for an hour. The mixture was extracted with 0.1 mL of ethyl acetate and dried with sodium sulfate. Farnesol was determined with GC. A no-phosphatase control was included in each assay.

Detailed Description Text - DETX (197):

Approximately 5 .mu.g of purified erg9.DELTA.::HIS3 DNA was used to transform diploid strain CJ3A.times.CJ3B (a/.alpha., ura3/ura3, his3/his3, leu2/leu2, trp1/trp1, upc2/upc2, obtained from Dr. L. Parks, N.C. State University, Raleigh, N.C.) using the lithium acetate transformation procedure described in Gietz, et al. (1995). CJ3A.times.CJ3B is homozygous for the upc2 mutation. This mutation allows sterol uptake under aerobic conditions (Lewis, et al., 1988). It was believed that the upc2 mutation would allow the easy production of a haploid strain carrying a mutation in the erg9 gene. The histidine auxotrophy was necessary to select for strains carrying the plasmids that contain the functional HIS3 gene.

Detailed Description Text - DETX (199):

Hundreds of transformants were obtained. These HIS.sup.+ cells were then patched onto SC-His and allowed to grow for two more days. The transformants were then patched onto sporulation medium (Sherman, et. al, 1986). Sporulation medium contains (per liter of distilled water): 1% potassium acetate, 0.1% Bacto yeast extract, 0.05% dextrose and 2% Bacto agar. The patches were then allowed to grow and sporulate for 3-5 days. A portion of the cells was removed

and placed in a solution of lyticase to digest the ascus cell wall. The cells were then spread in a thin line onto a YPD+2 mg/L ergosterol (YPDE) plate. The sporulated diploid cells form tetrads containing four spores, and the individual spores were separated using a micromanipulator. These individual haploid spores will germinate with each containing a single copy of the chromosomes.

Detailed Description Text - DETX (224):

HMG CoA reductase has been proposed to be the key enzyme regulating the flow of carbon through the isoprenoid pathway. In S. cerevisiae, two genes, HSG1 and HMG2, code for the two isozymes of HMG CoA reductase, designated HMGIp and HMG2p. Regulation of HMG CoA reductase is achieved through a combination of transcriptional and translational regulation as well as protein degradation. The segments of the HMG1 and HMG2 genes that encode the catalytic domains of the HMG1p and HMG2p isozymes have been cloned under transcriptional control of the strong promoter from the GPD (glyceraldehyde-3-phosphate dehydrogenase) gene. The plasmids containing these constructions (pRH127-3 and pRH124-31, containing the catalytic domains of HMG1p and HMG2p, respectively) were obtained from R. Hampton, U.C. San Diego. Strains of S. cerevisiae overexpressing the catalytic domain of HMGIp were reported to have an elevated flow of carbon through the isoprenoid pathway. This increased carbon flow was manifested as squalene and ergosterol overproduction (Donald, K. A. G., Hampton, R. Y., and Fritz, I. B., 1997, Effects of Overproduction of the Catalytic Domain of 3-Hydroxy-3-Methylglutaryl Coenzyme A Reductase on Squalene Synthesis in Saccharomyces cerevisiae. Applied and Environmental Microbiology 63:3341-3344).

Detailed Description Text - DETX (226):

In order to confirm that overexpressing HMG CoA reductase would result in increased carbon flow through the isoprenoid pathway in the strains derived from ATCC 28383, similar to other strains of S. cerevisiae (Donald, et. al., 1997), the strain SWY5, a ura3 mutant of the parental strain ATCC 28383, and SWE23-E9, the erg9 repaired version of EMS9-23 (strains described in Example 1.G. above), were transformed with plasmids pRH127-3 and pRH124-31. Transformations were performed using the LiOAc procedure (Gietz, et. al., 1995). Control strains carrying the empty vector YEp352 were also constructed. Representative transformants were tested for squalene and ergosterol production in a shake flask experiment. Cells were grown in 50 ml SCE-ura medium at 30.degree. C., with shaking at 180 rpm for 48 hours. At 24 hours, 10 ml of the culture was withdrawn, and the cells harvested for HMGCoA reductase assays as described in Example I.D. At the end of the 48-hour growth period, an aliquot of the cultures was used to inoculate flasks containing 50 ml YPD medium such that the starting OD.sub.600 of the cultures was 0.1. The cultures in YPD medium were grown for 72 hours at 30.degree. C., with shaking at 180 rpm, and then analyzed for dry cell weight, squalene and ergosterol accumulation. The extraction method used for this analysis was as follows. Ten ml of the culture was pelleted by centrifugation at 1,500.times.g for 10 minutes. The cell pellet was resuspended in 1 ml of deionized water, and the cells were repelleted. Cell pellets were resuspended in 1 ml of deionized water and disrupted by agitation with zirconium silicate beads (0.5 mm diameter). The broken cell suspension was saponified with 2.5 ml of a 0.2% solution of pyrogallol (in methanol) and 1.25 ml of 60% potassium hydroxide solution at 75 degrees C. for 1.5 hours. The samples were then extracted with 5 ml of hexane, vortexed for 3 minutes and centrifuged at 1,000.times.g for 20 minutes to separate the phases. The hexane layer was then analyzed by GC-MS as described in Example 1.B.

Detailed Description Text - DETX (227):

The data from this experiment are shown in Table 10. Compared to the control strains carrying only the Yep352 vector, the strains that overexpress the catalytic domains of either HMG1p or HMG2p contained high levels of HMGCoA reductase activity and elevated levels of squalene. However, neither of the strains overexpressing HMG1p or HMG2p contained significantly increased levels of ergosterol. Nevertheless, these data show that increasing the activity of HMG CoA reductase in the MBNA1-13 strain lineage increases the carbon flow through the isoprenoid pathway.

Detailed Description Text - DETX (229):

Having shown that amplification of HMG1p or HMG2p increased carbon flow to squalene, whether overexpression of HMG1 or HMG2 would increase farnesol production in a strain carrying the erg9 mutation was tested. Strain SWE23-.DELTA.E91 (described in Example 1.G.) was transformed with plasmids pRH127-3 or pRH124-31. Transformation of SWE23-.DELTA.E91 was accomplished using the LiOAc transformation procedure (Gietz et al, 1996). Approximately 2.5 .mu.g of pRH127-3 or pRH124-31 DNA were used in each transformation. Transformants were selected on SCE-ura plates, and several were chosen and restreaked for purification on SCE-ura plates. To test the effect of amplified HMG CoA reductase on farnesol production, representative transformants were grown for 48 hours in liquid SCE-ura medium. A control strain, SWE23-.DELTA.E91/YEp352, was also included in the experiment. At the end of the 48 hours growth period, aliquots of the cultures were used to inoculate flasks containing 50 ml of YPDE medium such that the initial OD.sub.600 was 0.5. These cultures were grown for 72 hours at 30.degree. C., with shaking at 180 rpm. At the end of the incubation period, samples were analyzed for dry cell weight and farnesol concentration. To confirm that the HMG CoA reductase genes were being overexpressed in the transformants, 20 ml of the cultures grown in SCE-ura were harvested by centrifugation at 1500.times.g for 10 minutes, and HMG CoA reductase activity measured using the permeabilized cell method described in Example I.D. The data from this experiment are shown in Table 11.

Detailed Description Text - DETX (247):

Strain EMS9-23 (ura3, erg9, described in Example 1.G.) was transformed with the plasmids described above using the LiOAc method, and transformants were selected on SCE-ura plates. Transformants were picked and restreaked on SCE-ura plates for purification. Representative transformants were tested for GG production. The strains were grown at 30.degree. C. for 48 hours in liquid SCE-ura medium, then used to inoculate flasks containing YPDE medium, such that the initial OD.sub.600 was 0.5. These cultures were incubated at 30.degree. C. with shaking for 72 hours and analyzed for dry cell weight, farnesol and GG levels. A second set of flasks containing 20 ml of SCE-ura medium was also inoculated from the same starting cultures and was grown for 48 hours. The cells from these flasks were harvested, washed with 10 ml 50 mM BisTris-Propane buffer, pH 7.0, and repelleted. The cell pellets were then used to prepare permeabilized cell suspensions for GGPP synthase assays. The cells were permeabilized by resuspending in 1 ml of 50 mM Bis-Tris-Propane buffer, pH 7.0, containing 0.1% Triton X100, and frozen at 80.degree. C. until needed. After thawing, the permeabilized cells were used for GGPP synthase assays. The GGPP synthase assay mixture contained 0.05M Bis-Tris propane buffer, pH 7.0 (X .mu.l), O.1M dithiothreitol (1 .mu.l), 1 mg/ml FPP (5 .mu.l), 1 mg/ml IPP (5 .mu.l) and permeabilized cells (Y .mu.1), where X+Y=87 .mu.l. A control reaction was included in which the FPP and IPP were omitted. The assay mixtures were incubated at 37.degree. C. for 20 minutes. 0. 1 ml of

2.times.glycine buffer (0.2M glycine, 2 mM MgCl.sub.2, 2 mM ZnCl.sub.2, pH 10.4) and 63 units of alkaline phosphatase (Sigma Chemical Co., St. Louis, Mo.) were added, and the mixtures were incubated for 60 minutes at 37.degree. C. The mixtures were then extracted with 0.2 ml 1:1 hexane:ethyl acetate, and analyzed for GG by GC/MS. GGPP synthase activity is expressed as nmol GGPP formed/min/mg protein.

Detailed Description Text - DETX (255):

To determine the effects of FPP synthase overexpression in erg9 mutant strains, representative transformants constructed as described above were grown in shake flask cultures and compared for growth, farnesol production, GG production and FPP synthase and GGPP synthase activities. The strains were grown with shaking at 30.degree. C. for 48 hours in liquid SCE-ura medium, then used to inoculate flasks containing YPDE medium, such that the initial OD.sub.600 was 0.5. These cultures were incubated at 30.degree. C. with shaking for an additional 72 hours and analyzed for dry cell weight, farnesol and GG levels. A second set of flasks containing 20 ml of SCE-ura medium was also inoculated from the same starting cultures and was grown for 48 hours. The cells from these flasks were harvested, washed with 10 ml 50 mM BisTris-Propane buffer, pH 7.0, and repelleted. The cell pellets were then used to prepare permeabilized cell suspensions for FPP and GGPP synthase assays. The cells were permeabilized by resuspending in 1 ml of 50 mM Bis-Tris-Propane buffer, pH 7.0, containing 0.1% Triton X100, and frozen at 80.degree. C. until needed. After thawing, the permeabilized cells were used for assays. The GGPP synthase assay mixture was the same as described in Example 5. The FPP synthase assay mixture contained 0.05 M Bis-Tris propane buffer, pH 7.0 (X .mu.l), 0.1M dithiothreitol (1 .mu.l), 0.5M MgCl.sub.2 (2 .mu.l), 1 mg/ml IPP (6 .mu.l), 1 mg/ml geranyldiphosphate (GPP) (6 .mu.l), and permeabilized cells (Y .mu.l), where X+Y=85 .mu.l. A control reaction was included in which the IPP and GPP were omitted. The assay mixtures were incubated at 37.degree. C. for 15 minutes. 0. 1 ml of 2.times.glycine buffer (0.2M glycine, 2 mM MgCl.sub.2, 2 mM ZnCl.sub.2, pH 10.4) and 63 units of alkaline phosphatase (Sigma Chemical Co., St. Louis, Mo.) were added, and the mixtures were incubated for 60 minutes at 37.degree. C. The mixtures were then extracted with 0.2 ml 1:1 hexane:ethyl acetate, and analyzed for Farnesol by GC/MS. FPP synthase activity is expressed as nmol FPP formed/min/mg protein.

Detailed Description Text - DETX (291):

These data support the idea that strains with elevated levels of HMG CoA reductase produce more farnesol than a strain with normal levels of HMG CoA reductase. The data also indicate that a strain containing eight integrated copies of the HMG2cat gene produce essentially as much farnesol as a strain that carries more than 20 extrachromosomal copies of the HMG2cat gene as is the case with strain SW23B/pRH124-31. A strain containing a single integrated copy of the HMG2 cat gene produced more farnesol than the control strain, but slightly less than strains with more copies of the HMG2cat gene. In addition, the strains containing elevated HMG CoA reductase levels accumulated mevalonate in the culture while the strains with normal levels of HMG CoA reductase did not. This suggests that a step downstream of HMG CoA reductase limits carbon flux in the pathway once the HMG CoA reductase enzyme activity has been elevated (see Example 10). Carbon flux through the pathway is restricted by the activity of one of the enzymes downstream of HMG CoA reductase resulting in mevalonate accumulation in the medium.

Detailed Description Text - DETX (293):

This example shows the effects of over-expression of multiple isoprenoid

pathway genes in a strain that has an erg9 mutation and elevated levels of HMG CoA reductase.

Detailed Description Text - DETX (294):
As shown in Examples 4 and 9, elevation of HMG CoA reductase levels led to higher carbon flux through the isoprenoid/sterol pathway. Over-expression of other isoprenoid pathway genes in strains containing amplified HMG CoA reductase may further increase carbon flux through this pathway. Amplification of isoprenoid pathway genes in strains that have elevated levels of HMG CoA reductase as well as a defective erg9 gene may result in further elevation of farnesol levels. Also, amplification of isoprenoid pathway genes may result in further elevation of GG levels in strains that have a defective erg9 gene and have elevated levels of HMG CoA reductase and GGPP synthase.

Detailed Description Text - DETX (295):
To test these ideas, plasmids were constructed that allowed for the over-expression of multiple isoprenoid pathway genes. One of the plasmids provided for the over-expression of mevalonate kinase, phosphomevalonate kinase, and diphosphomevalonate decarboxylase, coded by the ERG12, ERG8, and ERG19 genes respectively. This plasmid is referred to as pSW77-69, and was constructed from DNA fragments obtained from a number of plasmids containing single isoprenoid pathway genes. The construction of those plasmids is described first.

Detailed Description Text - DETX (316):
These data show that a strain which over-expresses HMG CoA reductase accumulates the isoprenoid pathway intermediate mevalonate in the culture medium. Since mevalonate accumulation is not observed in strains with normal levels of HMG CoA reductase, this demonstrates that carbon flux through the isoprenoid pathway has been increased by HMG CoA reductase amplification to the point where another step subsequent to HMG CoA reductase limits the conversion of pathway intermediates. Furthermore, over-expression of the first three enzymes in the isoprenoid pathway, namely acetoacetyl CoA thiolase, HMG CoA synthase, and HMG CoA reductase (coded by ERG 13 , ERG10, and HMG2cat, respectively) led to even higher accumulation of mevalonate in the medium. This demonstrates that carbon flux into the isoprenoid pathway has been increased further by amplification of the first three steps, and emphasizes that one of the enzymes downstream of HMG CoA reductase limits conversion of isoprenoid pathway intermediates. Since mevalonate serves as a precursor to farnesol and GG, the modifications described above can be used to increase carbon flux into the isoprenoid pathway, and, if the mevalonate can be more efficiently metabolized, can lead to increased farnesol and GG accumulation.

Detailed Description Text - DETX (326):
In order to construct strains of E. coli that accumulate elevated levels of farnesol, plasmids were constructed for the over-expression in E. coli of the genes listed above. In some cases, additional genes adjacent to the known isoprenoid pathway gene were included in the cloned DNA.

Detailed Description Text - DETX (327):
The idi gene coding for IPP isomerase was PCR amplified using the two oligonucleotides listed below and genomic DNA isolated from E. coli strain W3110. The oligonucleotides used to amplify the idi gene contained sequences corresponding to bases #42309 to #42335 and the reverse complement of #43699 to

#43726 of Gene Bank Accession #U28375. The lower case letters are used for bases that were altered to create restriction endonuclease recognition sites, which are indicated in parentheses following the oligonucleotide sequence. A natural EcoRI site was used in VE145-5.

Detailed Description Text - DETX (335):

The resulting PCR product was digested with EcoRI and HindIII, and ligated into pET24d(+) (Novagen) so that expression of the ctrE gene was controlled by the T7 promoter. The resulting plasmid is referred to as pKL19-63. This plasmid can be transformed into E. coli strains such as BL21(DE3) (available from Novagen) which contains an IPTG inducible gene coding for T7 polymerase. This allows IPTG induction of the crtE gene in this strain. The T7 promoter/crtE gene fusion can be cut from pKL19-63 using BglII and HindIII, and ligated into BamHI, HindIII digested pACYC184 (Accession #X06403) to construct a plasmid relying on chloramphenicol resistance for selection. This plasmid contains the p15A origin of replication and would be compatible with plasmids containing the ColE1 origin of replication such as the clones carrying the E. coli isoprenoid pathway genes described above. These latter plasmids confer ampicillin resistance, and so E. coli transformants can be obtained that carry both the crtE plasmid and the plasmid containing the dxs, dxr, idi, and ispA genes by transforming E. coli with both plasmids and selecting for resistance to chloramphenicol and ampicillin. Therefore, strains of E. coli BL21(DE3) will be obtained that contain plasmids for over-expression deoxyxylulose-5-phosphate synthase, deoxyxylulose-5-phosphate reductoisomerase, IPP isomerase, FPP synthase, and GGPP synthase. These strains will be tested for production of GG in shake flask and fermentation experiments.

Detailed Description Paragraph Table - DETL (39):

TABLE 17 Solvent Farnesol (ng) Hexane 3273 Chloroform 2262 Ethyl acetate 3549 N-heptane 3560 Hexadecane Not determined* Dodecane 3529 Toluene 1055 Carbon tetrachloride 2023 Isobutyl Alcohol 2892 1-Octanol 1814 *Solvent peak coeluted with farnesol, making quantification of farnesol impossible using this method.

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PARENT-CASE:

REFERENCE TO RELATED APPLICATION

This application claims priority from U.S. Provisional Application Serial No. 60/091,868, filed Jul. 6, 1998, entitled "Method of Vitamin Production."

US-CL-CURRENT: 435/125

ABSTRACT:

The invention provides a method of producing .alpha.-tocopherol and .alpha.-tocopheryl esters. The method comprises using a biological system to produce farnesol or geranylgeraniol. Then, the farnesol or geranylgernaiol is chemically converted into .alpha.-tocopherol or an .alpha.-tocopheryl ester.

29 Claims, 18 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 18

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Detailed Description Text - DETX (9):

Suitable biological systems for producing farnesol and GG include prokaryotic and eukaryotic cell cultures and cell-free systems. Preferred biological systems include fungal, bacterial and microalgal systems. More preferred biological systems are fungal cell cultures, more preferably a yeast cell culture, and most preferably a Saccharomyces cerevisiae cell culture. Fungi are preferred since they have a long history of use in industrial processes and can be manipulated by both classical microbiological and genetic engineering techniques. Yeast, in particular, are well-characterized genetically. Indeed, the entire genome of S. cerevisiae has been sequenced, and the genes coding for enzymes in the isoprenoid pathway have already been cloned. Also, S. cerevisiae grows to high cell densities, and amounts of

squalene and ergosterol (see FIG. 1) up to 16% of cell dry weight have been reported in genetically-engineered strains. For a recent review of the isoprenoid pathway in yeast, see Parks and Casey, Annu. Rev. Microbiol. 49:95-116 (1995).

Detailed Description Text - DETX (19):

A further embodiment of the present invention is the use of a microorganism which has been genetically modified to increase the action of HMG-CoA reductase. It should be noted that reference to increasing the action of HMG-CoA reductase and other enzymes discussed herein refers to any genetic modification in the microorganism in question which results in increased functionality of the enzymes and includes higher activity of the enzymes, reduced inhibition or degradation of the enzymes and overexpression of the enzymes. For example, gene copy number can be increased, expression levels can be increased by use of a promoter that gives higher levels of expression than that of the native promoter, or a gene can be altered by genetic engineering or classical mutagenesis to increase the activity of an enzyme. One of the key enzymes in the mevalonate-dependent isoprenoid biosynthetic pathway is HMG-CoA reductase which catalyzes the reduction of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA). This is the primary rate-limiting and first irreversible step in the pathway, and increasing HMG-CoA reductase activity leads to higher yields of squalene and ergosterol in a wild-type strain of S. cerevisiae, and farnesol in an erg9 strain. One mechanism by which the action of HMG-CoA reductase can be increased is by reducing inhibition of the enzyme, by either genetically modifying the enzyme or by modifying the system to remove the inhibitor. For instance, both sterol and non-sterol products of the isoprenoid pathway feedback inhibit this enzyme (see, e.g., Parks and Casey, Annu. Rev. Microbiol. 49:95-116 (1995). Alternatively or in addition, the gene(s) coding for HMG-CoA reductase can be altered by genetic engineering or classical mutagenesis techniques to decrease or prevent inhibition. Also, the action of HMG-CoA reductase can be increased by increasing the gene copy number, by increasing the level of expression of the HMG-CoA reductase gene(s), or by altering the HMG-CoA reductase gene(s) by genetic engineering or classical mutagenesis to increase the activity of the enzyme. See U.S. Pat. No. 5,460,949, the entire contents of which are incorporated herein by reference. For example, truncated HMG-CoA reductases have been produced in which the regulatory domain has been removed and the use of gene copy numbers up to about six also gives increased activity. Id. See also, Downing et al., Biochem. Biophys. Res. Commun., 94, 974-79 (1980) describing two yeast mutants having increased levels of HMG-CoA reductase. Two isozymes of HMGCoA reductase, encoded by the HMG1 and HMG2 genes, exist in S. cerevisiae. The activity of these two isozymes is regulated by several mechanisms including regulation of transcription, regulation of translation, and for Hmg2p, degradation of the enzyme in the endoplasmic reticulum (Hampton and Rine, 1994; Donald, et. al. 1997). In both Hmglp and Hmg2p, the catalytic domain resides in the COOH terminal portion of the enzyme, while the regulatory domain resides in the membrane spanning NH.sub.2 -terminal region. It has been shown that overexpression of just the catalytic domain of Hmglp in S. cerevisiae increases carbon flow through the isoprenoid pathway, resulting in overproduction of squalene (Saunders, et. al. 1995; Donald, et. al., 1997). The present inventors have expressed the catalytic domain of the S. cerevisiae Hmg2p in strains having a normal (i.e., unblocked) isoprenoid pathway and observed a significant increase in the production of squalene. Furthermore, overexpression of the catalytic domain of Hmg2p resulted in increased farnesol production in an erg9 mutant, and increased farnesol and GG production in an erg9 mutant overexpressing GGPP synthase, grown in fermentors.

Detailed Description Text - DETX (21):

A further embodiment of the present invention is the use of a microorganism which has been genetically modified to increase the action of GGPP synthase. Genes coding for this enzyme from a variety of sources, including bacteria, fungi, plants, mammals, and archaebacteria, have been identified. See, Brinkhaus et al., Arch. Biochem. Biophys., 266, 607-612 (1988); Carattoli et al., J. Biol. Chem., 266, 5854-59 (1991); Chen et al., J. Biol. Chem., 268, 11002-11007 (1993); Dogbo et al., Biochim. Biophys. Acta, 920, 140-148 (1987); Jiang et al., J. Biol. Chem., 270, 21793-99 (1995); Kuntz al., Plant J., 2, 25-34 (1992); Laferriere, et al., Biochim. Biophys. Acta, 1077, 167-72 (1991); Math et al., Proc. Natl. Acad. Sci. USA, 89, 6761-64 (1992); Ohnuma et al., J. Biol. Chem., 269, 14792-97 (1994); Sagami et al., Arch. Biochem. Biophys., 297, 314-20 (1992); Sagami et al., J. Biol. Chem., 269, 20561-66 (1994); Sandmann et al., J. Photochem. Photobiol. B: Biol., 18, 245-51 (1993); Scolnik et al., Plant Physiol., 104, 1469-70 (1994); Tachibana et al., Biosci. Biotech. Biochem., 7, 1129-33 (1993); Tachibana et al., J. Biochem., 114, 389-92 (1993); Wiedemann et al., Arch. Biochem. Biophys., 306, 152-57 (1993). Some organisms have a bifunctional enzyme which also serves as an FPP synthase, so it is involved in the overall conversion of IPP and DMAPP to FPP to GGPP (see FIG. 1). Some enzymes, such as those found in plants, have relaxed specificity, converting IPP and DMAPP to GGPP (see FIG. 1). Genetic modifications of GGPP synthase, as used herein, encompass engineering a monofunctional GGPP synthase or a bifunctional FPP/GGPP synthase to enhance the GGPP synthase activity component of the enzyme. A preferred GGPP synthase gene is the BTS1 gene from S. cervisiae. The BTS1 gene and its isolation are described in Jiang et al., J. Biol. Chem., 270, 21793-99 (1995) and U.S. Pat. No. 5,912,154, filed on Dec. 6, 1996, the complete disclosure of which incorporated herein by reference. However, GGPP synthases of other hosts can be used, and the use of the bifunctional GGPP syntheses may be particularly advantageous in terms of channeling carbon flow through FPP to GGPP, thereby avoiding loss of FPP to competing reactions in the cell.

Detailed Description Text - DETX (34):

Blocking Pathwavs that Compete for FPP or GGPP. In yeast, FPP is a branch point intermediate leading to the biosynthesis of sterols, heme, dolichol, ubiquinone, GGPP and farnesylated proteins. In E. coli, FPP serves as the substrate for octaprenyl pyrophosphate synthase in the pathway leading to ubiquinone. In bacteria that synthesize carotenoids, such as Erwina uredovora, FPP is converted to GGPP by GGPP synthase in the first step leading to the carotenoids. To increase the production of farnesol or GG, it is desirable to inactivate genes encoding enzymes that use FPP or GGPP as substrate, or to reduce the activity of the enzymes themselves, either through mutation or the use of specific enzyme inhibitors (as was discussed above for squalene synthase). In S. cerevisiae, for example, it may be advantageous to inactivate the first step in the pathway from FPP to heme, in addition to inactivating ERG9. As discussed earlier, in E. coli, partial or complete inactivation of the octaprenyl pyrophosphate synthase could increase the availability of FPP for conversion of farnesol. Finally, in bacteria that produce carotenoids, such as Erwina uredovora, elimination of GGPP synthase can increase the level of FPP for conversion of farnesol, while inactivating or reducing the activity of phytoene synthase (the crtB gene product) can increase the level of GGPP available for conversion to GG.

Detailed Description Text - DETX (43):

Sources of assimilable carbon which can be used in a suitable fermentation medium include, but are not limited to, sugars and their polymers, including, dextrin, sucrose, maltose, lactose, glucose, fructose, mannose, sorbose,

arabinose and xylose; fatty acids; organic acids such as acetate; primary alcohols such as ethanol and n-propanol; and polyalcohols such as glycerine. Preferred carbon sources in the present invention include monosaccharides, disaccharides, and trisaccharides. The most preferred carbon source is glucose.

Detailed Description Text - DETX (78):

FIG. 2 illustrates a particularly preferred embodiment of vitamin E synthesis from geranylgeraniol. The hydroxy group of geranylgeraniol 2-1 is protected as isobutyrate ester or other bulky ester using the corresponding acid or its derivative such as anhydride or acyl chloride. Geranylgeranylisobutyrate 2-2 is then selectively hydrogenated using a metal catalyst such as palladium on carbon to produce phytylisobutyrate 2-3. Hydrogenation of geranylgeranylisobutyrate 2-2 reduces the second, third and fourth olefin moieties without affecting the first olefin moiety. It is believed that the steric bulk of isobutyrate decreases the rate of hydrogenation of the first olefin moiety in protected geranylgeraniol, thus leading to a selective hydrogenation of olefin moieties. The hydroxy protecting group is then removed by hydrolysis under a basic condition to provide phytol 2-4. Reacting phytol 2-4 with 2,3,5-trimethyl hydroquinone 2-5 in the presence of an acid then results in a formation of vitamin E 2-6. Alternatively, phytylisobutyrate 2-3 can be deprotected and reacted with 2,3,5-trimethyl hydroquinone 2-5 in a single step by an acid catalyst to form vitamin E 2-6. Vitamin E 2-6 can further be transformed to a protected vitamin E such as vitamin E acetate 2-7 by acetylation of the free hydroxy moiety.

Detailed Description Text - DETX (80):

The step of selectively oxidizing the 2,3-carbon-carbon double bond in geranylgeraniol to produce geranylgeraniol-2,3-epoxide is carried out with any reagent known to selectively epoxidize the olefin functionality of allylic alcohols, such as combinations of tert-butyl hydroperoxide and vanadium or molybdenum catalysts as taught by the method of Sharpless and Michaelson, J. Am. Chem. Soc. 95, 6136 (1973). Hydrogen peroxide or other alkyl hydroperoxides could replace tert-butyl hydroperoxide, and inert solvents such as aromatic hydrocarbons, aliphatic hydrocarbons, cycloaliphatic hydrocarbons or aliphatic esters may be used. The term "aliphatic hydrocarbon" is used to include straight or branched chain alkanes having five to eight carbon atoms. Heptane is the most preferred aliphatic hydrocarbon. The term "cycloaliphatic hydrocarbon" is used to include C.sub.5 -C.sub.8 cycloalkanes and these substituted with one or more C.sub.1 -C.sub.4 alkyl groups. The term "aromatic hydrocarbon" is used to include benzene and benzene substituted with one to three C.sub.1 -C.sub.4 alkyl groups. Toluene is the most preferred aromatic hydrocarbon. The term "aliphatic esters" is used to include aliphatic esters having three to nine carbon having the formula R.sub.1 CO.sub.2 R.sub.2 wherein R.sub.1 and R.sub.2 independently represent a straight or branched chain C.sub.1 -C.sub.4 alkyl group, with ethyl acetate being the preferred aliphatic ester.

Detailed Description Text - DETX (84):

Specific exemplary compounds of generic terms used herein which are suitable for use in the present invention are as follows. Typical aromatic solvents include benzene, toluene, and o, m or p-xylene or mixtures thereof. Toluene is the preferred aromatic solvent. Typical aliphatic solvents include n-pentane, n-hexane, n-heptane and n-octane and isomers thereof, n-Heptane is the preferred aliphatic hydrocarbon. Typical cycloaliphatic hydrocarbons include cyclopentane, cyclohexane, methylcyclohexane and cycloheptane. Typical

aliphatic esters include methyl acetate, ethyl acetate, n-propyl acetate, n-butyl acetate, isopropyl acetate, methyl propionate, ethyl propionate, methyl butyrate and ethyl butyrate. Ethyl acetate is the preferred aliphatic ester. Typical alkanols and substituted alkanols include methanol, ethanol, n-propanol, isopropanol, n-butanol, isobutanol, 2-methoxyethanol, 2-ethoxyethanol and 2-isopropoxyethanol. Typical ethers include diethyl ether, diisopropyl ether, dibutyl ether, n-butyl methyl ether, t-butyl methyl ether and tetrahydrofuran. Typical useful Lewis acids include zinc chloride, aluminum chloride, boron trifluoride, ferric chloride and phosphoric acid. Zinc chloride is the preferred Lewis acid.

Detailed Description Text - DETX (124):

The mutants were assayed for squalene synthase activity. The squalene synthase assay involved incubation of a cell-free extract with FPP in the presence the reduced form of nicotinamide adenine dinucleotide phosphate, (NADPH), extraction into ethyl acetate, and squalene detection by GC. Specifically, 0.1 M Tris/HCl pH7 (X .mu.L), 0.1 M DTT (2 .mu.L), 0.1 M MgCl.sub.2 (10 .mu.L), 0.1 M NADPH (4 .mu.L), 1 mg/mL FPP (6 .mu.L), and cell-free extract (7000.times.g supernatant) (Y .mu.L), where X+Y=178 .mu.L to make 200 .mu.L total, were combined. This mixture was incubated in glass tubes at 37.degree. C. for 40 min. Then, it was extracted with 0.15 mL ethyl acetate. The extract was transferred to plastic vials and centrifuged at 15,000.times.g for 5 min. The ethyl acetate extract was analyzed using GC/MS.

Detailed Description Text - DETX (127):

For the FPP synthase assay, 0.1 M DTT (2 .mu.L), 0.1 M MgCl.sub.2 (2 .mu.L), 1 mg/mL IPP (6 .mu.L), 1 mg/mL GPP (6 .mu.L), cell-free extract (Y), and 0.1 M Tris/HCl (pH 7.0) (X .mu.L), where X+Y=84 .mu.L, were combined, and incubated at 37.degree. C. for 15 min. This mixture was then extracted twice with 0.3 mL hexane to remove pre-existing farnesol. Next, 0.1 mL of 2.times.glycine buffer (0.2 M glycine, 2 mM MgCl.sub.2, 2 mM Zn Cl.sub.2), pH 10.4, and 33 units of alkaline phosphatase were added, and the mixture was incubated at 37.degree. C. for an hour. The mixture was extracted with 0.1 mL of ethyl acetate and dried with sodium sulfate. Farnesol was determined with GC. A no-phosphatase control was included in each assay.

Detailed Description Text - DETX (204):

Approximately 5 .mu.g of purified erg9.DELTA.::HIS3 DNA was used to transform diploid strain CJ3A X CJ3B (a/.alpha., ura3/ura3, his3/his3, leu2/leu2, trp1/trp1, upc2/upc2, obtained from Dr. L. Parks, N.C. State University, Raleigh, N.C.) using the lithium acetate transformation procedure described in Gietz, et al. (1995). CJ3A X CJ3B is homozygous for the upc2 mutation. This mutation allows sterol uptake under aerobic conditions (Lewis, et al., 1988). It was believed that the upc2 mutation would allow the easy production of a haploid strain carrying a mutation in the erg9 gene. The histidine auxotrophy was necessary to select for strains carrying the plasmids that contain the functional HIS3 gene.

Detailed Description Text - DETX (206):

Hundreds of transformants were obtained. These HIS.sup.+ cells were then patched onto SC-His and allowed to grow for two more days. The transformants were then patched onto sporulation medium (Sherman, et. al, 1986). Sporulation medium contains (per liter of distilled water): 1% potassium acetate, 0.1% Bacto yeast extract, 0.05% dextrose and 2% Bacto agar. The patches were then allowed to grow and sporulate for 3-5 days. A portion of the cells was removed

and placed in a solution of lyticase to digest the ascus cell wall. The cells were then spread in a thin line onto a YPD+2 mg/L ergosterol (YPDE) plate. The sporulated diploid cells form tetrads containing four spores, and the individual spores were separated using a micromanipulator. These individual haploid spores will germinate with each containing a single copy of the chromosomes.

Detailed Description Text - DETX (234):

HMG CoA reductase has been proposed to be the key enzyme regulating the flow of carbon through the isoprenoid pathway. In S. cerevisiae, two genes, HMG1 and HMG2, code for the two isozymes of HMG CoA reductase, designated HMG1p and HMG2p. Regulation of HMG CoA reductase is achieved through a combination of transcriptional and translational regulation as well as protein degradation. The segments of the HMG1 and HMG2 genes that encode the catalytic domains of the HMG1p and HMG2p isozymes have been cloned under transcriptional control of the strong promoter from the GPD (glyceraldehyde-3-phosphate dehydrogenase) gene. The plasmids containing these constructions (pRH127-3 and pRH124-31, containing the catalytic domains of HMG1p and HMG2p, respectively) were obtained from R. Hampton, U. C. San Diego. Strains of S. cerevisiae overexpressing the catalytic domain of HMG1p were reported to have an elevated flow of carbon through the isoprenoid pathway. This increased carbon flow was manifested as squalene and ergosterol overproduction (Donald, K. A. G., Hampton, R. Y., and Fritz, I. B., 1997, Effects of Overproduction of the Catalytic Domain of 3-Hydroxy-3-Methylglutaryl Coenzyme A Reductase on Squalene Synthesis in Saccharomyces cerevisiae. Applied and Environmental Microbiology 63:3341-3344).

Detailed Description Text - DETX (236):

In order to confirm that overexpressing HMG CoA reductase would result in increased carbon flow through the isoprenoid pathway in the strains derived from ATCC 28383, similar to other strains of S. cerevisiae (Donald, et. al., 1997), the strain SWY5, a ura3 mutant of the parental strain ATCC 28383, and SWE23-E9, the erg9 repaired version of EMS9-23 (strains described in Example 1.G. above), were transformed with plasmids pRH127-3 and pRH124-31. Transformations were performed using the LiOAc procedure (Gietz, et. al., 1995). Control strains carrying the empty vector YEp352 were also constructed. Representative transformants were tested for squalene and ergosterol production in a shake flask experiment. Cells were grown in 50 ml SCE-ura medium at 30.degree. C., with shaking at 180 rpm for 48 hours. At 24 hours, 10 ml of the culture was withdrawn, and the cells harvested for HMGCoA reductase assays as described in Example I.D. At the end of the 48-hour growth period, an aliquot of the cultures was used to inoculate flasks containing 50 ml YPD medium such that the starting OD.sub.600 of the cultures was 0.1. The cultures in YPD medium were grown for 72 hours at 30.degree. C., with shaking at 180 rpm, and then analyzed for dry cell weight, squalene and ergosterol accumulation. The extraction method used for this analysis was as follows. Ten ml of the culture was pelleted by centrifugation at 1,500.times.g for 10 minutes. The cell pellet was resuspended in 1 ml of deionized water, and the cells were repelleted. Cell pellets were resuspended in 1 ml of deionized water and disrupted by agitation with zirconium silicate beads (0.5 mm diameter). The broken cell suspension was saponified with 2.5 ml of a 0.2% solution of pyrogallol (in methanol) and 1.25 ml of 60% potassium hydroxide solution at 75 degrees C. for 1.5 hours. The samples were then extracted with 5 ml of hexane, vortexed for 3 minutes and centrifuged at 1,000.times.g for 20 minutes to separate the phases. The hexane layer was then analyzed by GC-MS as described in Example 1.B.

Detailed Description Text - DETX (237):

The data from this experiment are shown in Table 10. Compared to the control strains carrying only the Yep352 vector, the strains that overexpress the catalytic domains of either HMG1p or HMG2p contained high levels of HMGCoA reductase activity and elevated levels of squalene. However, neither of the strains overexpressing HMG1p or HMG2p contained significantly increased levels of ergosterol. Nevertheless, these data show that increasing the activity of HMG CoA reductase in the MBNA1-13 strain lineage increases the carbon flow through the isoprenoid pathway.

Detailed Description Text - DETX (239):

Having shown that amplification of HMG1p or HMG2p increased carbon flow to squalene, whether overexpression of HMG1 or HMG2 would increase farnesol production in a strain carrying the erg9 mutation was tested. Strain SWE23-.DELTA.E91 (described in Example 1.G.) was transformed with plasmids pRH127-3 or pRH124-31. Transformation of SWE23-.DELTA.E91 was accomplished using the LiOAc transformation procedure (Gietz et al, 1996). Approximately 2.5 .mu.g of pRH127-3 or pRH124-31 DNA were used in each transformation. Transformants were selected on SCE-ura plates, and several were chosen and restreaked for purification on SCE-ura plates. To test the effect of amplified HMG CoA reductase on farnesol production, representative transformants were grown for 48 hours in liquid SCE-ura medium. A control strain, SWE23-.DELTA.E91/YEp352, was also included in the experiment. At the end of the 48 hours growth period, aliquots of the cultures were used to inoculate flasks containing 50 ml of YPDE medium such that the initial OD.sub.600 was 0.5. These cultures were grown for 72 hours at 30.degree. C., with shaking at 180 rpm. At the end of the incubation period, samples were analyzed for dry cell weight and farnesol concentration. To confirm that the HMG CoA reductase genes were being overexpressed in the transformants, 20 ml of the cultures grown in SCE-ura were harvested by centrifugation at 1500.times.g for 10 minutes, and HMG CoA reductase activity measured using the permeabilized cell method described in Example I.D. The data from this experiment are shown in Table 11.

Detailed Description Text - DETX (271):

Strain EMS9-23 (ura3, erg9, described in Example 1.G.) was transformed with the plasmids described above using the LiOAc method, and transformants were selected on SCE-ura plates. Transformants were picked and restreaked on SCE-ura plates for purification. Representative transformants were tested for GG production. The strains were grown at 30.degree. C. for 48 hours in liquid SCE-ura medium, then used to inoculate flasks containing YPDE medium, such that the initial OD.sub.600 was 0.5. These cultures were incubated at 30.degree. C. with shaking for 72 hours and analyzed for dry cell weight, farnesol and GG levels. A second set of flasks containing 20 ml of SCE-ura medium was also inoculated from the same starting cultures and was grown for 48 hours. The cells from these flasks were harvested, washed with 10 ml 50 mM BisTris-Propane buffer, pH 7.0, and repelleted. The cell pellets were then used to prepare permeabilized cell suspensions for GGPP synthase assays. The cells were permeabilized by resuspending in 1 ml of 50 mM Bis-Tris-Propane buffer, pH 7.0, containing 0.1% Triton X100, and frozen at 80.degree. C. until needed. After thawing, the permeabilized cells were used for GGPP synthase assays. The GGPP synthase assay mixture contained 0.05M Bis-Tris propane buffer, pH 7.0 (X .mu.l), 0.1M dithiothreitol (1 .mu.l), 1 mg/ml FPP (5 .mu.l), 1 mg/ml IPP (5 .mu.l) and permeabilized cells (Y .mu.l), where X+Y=87 .mu.l. A control reaction was included in which the FPP and IPP were omitted. The assay mixtures were incubated at 37.degree. C. for 20 minutes. 0.1 ml of

2.times.glycine buffer (0.2M glycine, 2 mM MgCl.sub.2, 2 mM ZnCl.sub.2, pH 10.4) and 63 units of alkaline phosphatase (Sigma Chemical Co., St. Louis, Mo.) were added, and the mixtures were incubated for 60 minutes at 37.degree. C. The mixtures were then extracted with 0.2 ml 1:1 hexane:ethyl acetate, and analyzed for GG by GC/MS. GGPP synthase activity is expressed as nmol GGPP formed/min/mg protein.

Detailed Description Text - DETX (281):

To determine the effects of FPP synthase overexpression in erg9 mutant strains, representative transformants constructed as described above were grown in shake flask cultures and compared for growth, farnesol production, GG production and FPP synthase and GGPP synthase activities. The strains were grown with shaking at 30.degree. C. for 48 hours in liquid SCE-ura medium, then used to inoculate flasks containing YPDE medium, such that the initial OD.sub.600 was 0.5. These cultures were incubated at 30.degree. C. with shaking for an additional 72 hours and analyzed for dry cell weight, farnesol and GG levels. A second set of flasks containing 20 ml of SCE-ura medium was also inoculated from the same starting cultures and was grown for 48 hours. The cells from these flasks were harvested, washed with 10 ml 50 mM BisTris-Propane buffer, pH 7.0, and repelleted. The cell pellets were then used to prepare permeabilized cell suspensions for FPP and GGPP synthase assays. The cells were permeabilized by resuspending in 1 ml of 50 mM Bis-Tris-Propane buffer, pH 7.0, containing 0.1% Triton X100, and frozen at 80.degree. C. until needed. After thawing, the permeabilized cells were used for assays. The GGPP synthase assay mixture was the same as described in Example 5. The FPP synthase assay mixture contained 0.05 M Bis-Tris propane buffer, pH 7.0 (X .mu.l), 0.1M dithiothreitol (1 .mu.l), 0.5M MgCl.sub.2 (2 .mu.l), 1 mg/ml IPP (6 .mu.l), 1 mg/ml geranyldiphosphate (GPP) (6 .mu.l), and permeabilized cells (Y .mu.l), where X+Y=85 .mu.l. A control reaction was included in which the IPP and GPP were omitted. The assay mixtures were incubated at 37.degree. C. for 15 minutes. 0.1 ml of 2.times. glycine buffer (0.2M glycine, 2 mM MgCl.sub.2, 2 mM ZnCl.sub.2, pH 10.4) and 63 units of alkaline phosphatase (Sigma Chemical Co., St. Louis, Mo.) were added, and the mixtures were incubated for 60 minutes at 37.degree. C. The mixtures were then extracted with 0.2 ml 1:1 hexane:ethyl acetate, and analyzed for Farnesol by GC/MS. FPP synthase activity is expressed as nmol FPP formed/min/mg protein.

Detailed Description Text - DETX (333):

These data support the idea that strains with elevated levels of HMG CoA reductase produce more farnesol than a strain with normal levels of HMG CoA reductase. The data also indicate that a strain containing eight integrated copies of the HMG2cat gene produce essentially as much farnesol as a strain that carries more than 20 extrachromosomal copies of the HMG2cat gene as is the case with strain SW23B/pRH124-31. A strain containing a single integrated copy of the HMG2 cat gene produced more farnesol than the control strain, but slightly less than strains with more copies of the HMG2cat gene. In addition, the strains containing elevated HMG CoA reductase levels accumulated mevalonate in the culture while the strains with normal levels of HMG CoA reductase did not. This suggests that a step downstream of HMG CoA reductase limits carbon flux in the pathway once the HMG CoA reductase enzyme activity has been elevated (see Example 15). Carbon flux through the pathway is restricted by the activity of one of the enzymes downstream of HMG CoA reductase resulting in mevalonate accumulation in the medium.

Detailed Description Text - DETX (335):

This example shows the effects of over-expression of multiple isoprenoid

pathway genes in a strain that has an erg9 mutation and elevated levels of HMG CoA reductase.

Detailed Description Text - DETX (336):
As shown in Examples 4 and 14, elevation of HMG CoA reductase levels led to higher carbon flux through the isoprenoid/sterol pathway. Over-expression of other isoprenoid pathway genes in strains containing amplified HMG CoA reductase may further increase carbon flux through this pathway. Amplification of isoprenoid pathway genes in strains that have elevated levels of HMG CoA reductase as well as a defective erg9 gene may result in further elevation of farnesol levels. Also, amplification of isoprenoid pathway genes may result in further elevation of GG levels in strains that have a defective erg9 gene and have elevated levels of HMG CoA reductase and GGPP synthase.

Detailed Description Text - DETX (337):
To test these ideas, plasmids were constructed that allowed for the over-expression of multiple isoprenoid pathway genes. One of the plasmids provided for the over-expression of mevalonate kinase, phosphomevalonate kinase, and diphosphomevalonate decarboxylase, coded by the ERG12, ERG8, and ERG19 genes respectively. This plasmid is referred to as pSW77-69, and was constructed from DNA fragments obtained from a number of plasmids containing single isoprenoid pathway genes. The construction of those plasmids is described first.

Detailed Description Text - DETX (358):
These data show that a strain which over-expresses HMG CoA reductase accumulates the isoprenoid pathway intermediate mevalonate in the culture medium. Since mevalonate accumulation is not observed in strains with normal levels of HMG CoA reductase, this demonstrates that carbon flux through the isoprenoid pathway has been increased by HMG CoA reductase amplification to the point where another step subsequent to HMG CoA reductase limits the conversion of pathway intermediates. Furthermore, over-expression of the first three enzymes in the isoprenoid pathway, namely acetoacetyl CoA thiolase, HMG CoA synthase, and HMG CoA reductase (coded by ERG 13, ERG10, and HMG2cat, respectively) led to even higher accumulation of mevalonate in the medium. This demonstrates that carbon flux into the isoprenoid pathway has been increased further by amplification of the first three steps, and emphasizes that one of the enzymes downstream of HMG CoA reductase limits conversion of isoprenoid pathway intermediates. Since mevalonate serves as a precursor to farnesol and GG, the modifications described above can be used to increase carbon flux into the isoprenoid pathway, and, if the mevalonate can be more efficiently metabolized, can lead to increased farnesol and GG accumulation.

Detailed Description Text - DETX (368):
In order to construct strains of E. coli that accumulate elevated levels of farnesol, plasmids were constructed for the over-expression in E. coli of the genes listed above. In some cases, additional genes adjacent to the known isoprenoid pathway gene were included in the cloned DNA.

Detailed Description Text - DETX (369):
The idi gene coding for IPP isomerase was PCR amplified using the two oligonucleotides listed below and genomic DNA isolated from E. coli strain W3110. The oligonucleotides used to amplify the idi gene contained sequences corresponding to bases #42309 to #42335 and the reverse complement of #43699 to

#43726 of Gene Bank Accession #U28375. The lower case letters are used for bases that were altered to create restriction endonuclease recognition sites, which are indicated in parentheses following the oligonucleotide sequence. A natural EcoRI site was used in VE145-5.

Detailed Description Text - DETX (381):

The resulting PCR product was digested with EcoRI and HindIII, and ligated into pET24d(+) (Novagen) so that expression of the ctrE gene was controlled by the T7 promoter. The resulting plasmid is referred to as pKL19-63. This plasmid can be transformed into E. coli strains such as BL21(DE3) (available from Novagen) which contains an IPTG inducible gene coding for T7 polymerase. This allows IPTG induction of the crtE gene in this strain. The T7 promoter/crtE gene fusion can be cut from pKL19-63 using BglII and HindIII, and ligated into BamHI, HindIII digested pACYC184 (Accession #X06403) to construct a plasmid relying on chloramphenicol resistance for selection. This plasmid contains the p15A origin of replication and would be compatible with plasmids containing the ColE1 origin of replication such as the clones carrying the E. coli isoprenoid pathway genes described above. These latter plasmids confer ampicillin resistance, and so E. coli transformants can be obtained that carry both the crtE plasmid and the plasmid containing the dxs, dxr, idi, and ispA genes by transforming E. coli with both plasmids and selecting for resistance to chloramphenicol and ampicillin. Therefore, strains of E. coli BL21(DE3) will be obtained that contain plasmids for over-expression deoxyxylulose-5-phosphate synthase, deoxyxylulose-5-phosphate reductoisomerase, IPP isomerase, FPP synthase, and GGPP synthase. These strains will be tested for production of GG in shake flask and fermentation experiments.

Detailed Description Paragraph Table - DETL (24):

TABLE 17 Solvent Farnesol (ng) Hexane 3273 Chloroform 2262 Ethyl acetate 3549 N-heptane 3560 Hexadecane Not determined* Dodecane 3529 Toluene 1055 Carbon tetrachloride 2023 Isobutyl Alcohol 2892 1-Octanol 1814

* Solvent peak coeluted with farnesol, making quantification of farnesol impossible using this method.

Detailed Description Paragraph Table - DETL (25):

TABLE 17 Solvent Farnesol (ng) Hexane 3273 Chloroform 2262 Ethyl acetate 3549 N-heptane 3560 Hexadecane Not determined* Dodecane 3529 Toluene 1055 Carbon tetrachloride 2023 Isobutyl Alcohol 2892 1-Octanol 1814

* Solvent peak coeluted with farnesol, making quantification of farnesol impossible using this method.

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	6162	isoprenoid$1 or lycopene$1 or carotene$1 or astaxanthin$1 or phytoene$1 or isopentyl adj diphosphate or ipp	USPAT	OR	OFF	2004/02/16 15:36
L2	147	acetyl adj phosphate or acetylphosphate	USPAT	OR	OFF	2004/02/16 15:05
L3	219	acetyl adj phosphate or acetylphosphate	US-PGPUB; USPAT	OR	OFF	2004/02/16 15:05
L4	0	1 near4 (gene$1 or sequence$1) and (L2 or L3)	USPAT	OR	OFF	2004/02/16 15:07
L5	306498	acetate	USPAT	OR	OFF	2004/02/16 15:06
L6	122	1 near4 (gene$1 or sequence$1) and L5	US-PGPUB; USPAT	OR	OFF	2004/02/16 15:13
L7	0	1 near4 (gene$1 or sequence$1) and L2	US-PGPUB; USPAT	OR	OFF	2004/02/16 15:22
L8	1278906	induc$8 or regulat$8 or activat$8 or modulat$8	USPAT	OR	OFF	2004/02/16 15:13
L9	12	1 near4 (gene$1 or sequence$1) and (L5 same L8)	US-PGPUB; USPAT	OR	OFF	2004/02/16 15:13
L10	721	1 same (2 or 5)	US-PGPUB; USPAT	ADJ	OFF	2004/02/16 15:22
L11	2	1 near4 (gene$1 or sequence$1) same (2 or 5)	US-PGPUB; USPAT	OR	OFF	2004/02/16 15:24
L12	6	6 and carbon adj (flux or flow)	US-PGPUB; USPAT	OR	OFF	2004/02/16 15:35
L13	62775	promoter$1	USPAT	OR	OFF	2004/02/16 15:35
L14	507	(L2 or L5) same L13 same L8	USPAT	OR	OFF	2004/02/16 15:35
L15	17	1 and L14	US-PGPUB; USPAT	OR	OFF	2004/02/16 15:37

US-PAT-NO: 6680047

DOCUMENT-IDENTIFIER: US 6680047 B2

TITLE: Diagnostic/therapeutic agents

DATE-ISSUED: January 20, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Klaveness; Jo	Oslo	N/A	N/A	NO
Rongved; P.ang.l	Oslo	N/A	N/A	NO
H.o slashed.gset; Anders	Oslo	N/A	N/A	NO
Tolleshaug; Helge	Oslo	N/A	N/A	NO
Cuthbertson; Alan	Oslo	N/A	N/A	NO
Godal; Aslak	Oslo	N/A	N/A	NO
Hoff; Lars	Oslo	N/A	N/A	NO
Gogstad; Geir	Oslo	N/A	N/A	NO
Bryn; Klaus	Oslo	N/A	N/A	NO
N.ae butted.vestad; Anne	Oslo	N/A	N/A	NO
L.o slashed.vhaug; Dagfinn	Oslo	N/A	N/A	NO
Hellebust; Halldis	Oslo	N/A	N/A	NO
Solbakken; Magne	Oslo	N/A	N/A	NO

APPL-NO: 09/ 925715

DATE FILED: August 10, 2001

PARENT-CASE:

This application is a continuation application of pending U.S. application Ser. No. 08/959,206, filed Oct. 28, 1997 now U.S. Pat. No. 6,331,289, (of which the entire disclosure of the pending, prior application is hereby incorporated by reference) which has been allowed and the Issue Fee paid on Aug. 6, 2001, which claims benefit of three U.S. provisional applications serial Nos. 60/049,263, filed June 6, 1997, 60/049,264, filed Jun. 6, 1997 and 60/049,266, filed Jun. 7, 1997.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
GB	9622366	October 28, 1996
GB	9622369	October 28, 1996
GB	9702195	February 4, 1997
GB	9708265	April 24, 1997
GB	9711837	June 6, 1997
GB	9711839	June 6, 1997

US-CL-CURRENT: 424/9.52, 424/1.21 , 424/450 , 424/489 , 424/9.32 , 424/9.4

ABSTRACT:

Targetable diagnostic and/or therapeutically active agents, e.g. ultrasound contrast agents, comprising a suspension in an aqueous carrier liquid of a reporter comprising gas-containing or gas-generating material, said agent being capable of forming at least two types of binding pairs with a target.

30 Claims, 1 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 1

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Brief Summary Text - BSTX (75):

Representative and non-limiting examples of drugs useful in accordance with the invention include antineoplastic agents such as vincristine, vinblastine, vindesine, busulfan, chlorambucil, spiroplatin, cisplatin, carboplatin, methotrexate, adriamycin, mitomycin, bleomycin, cytosine arabinoside, arabinosyl adenine, mercaptopurine, mitotane, procarbazine, dactinomycin (antinomycin D), daunorubicin, doxorubicin hydrochloride, taxol, plicamycin, aminoglutethimide, estramustine, flutamide, leuprolide, megestrol acetate, tamoxifen, testolactone, trilostane, amsacrine (m-AMSA), asparaginase (L-asparaginase), etoposide, interferon a-2a and 2b, blood products such as hematoporphyrins or derivatives of the foregoing; biological response modifiers such as muramylpeptides; antifungal agents such as ketoconazole, nystatin, griseofulvin, flucytosine, miconazole or amphotericin B; hormones or hormone analogues such as growth hormone, melanocyte stimulating hormone, estradiol, beclomethasone dipropionate, betamethasone, cortisone acetate, dexamethasone, flunisolide, hydrocortisone, methylprednisolone, paramethasone acetate, prednisolone, prednisone, triamcinolone or fludrocortisone acetate; vitamins such as cyanocobalamin or retinoids; enzymes such as alkaline phosphatase or manganese superoxide dismutase; antiallergic agents such as amelexanox; inhibitors of tissue factor such as monoclonal antibodies and Fab fragments thereof, synthetic peptides, nonpeptides and compounds downregulating tissue factor expression; inhibitors of platelets such as, GPIa, GPIb and GPIIb-IIIa, ADP receptors, thrombin receptors, von Willebrand factor, prostaglandins, aspirin, ticlopidin, clopigogrel and reopro; inhibitors of coagulation protein targets such as: FIIa FVa, FVIIa, FVIIIA, FIXa, tissue factor, hepatins, hirudin, hirulog, argatroban, DEGR-rFVIIa and annexin V; inhibitors of fibrin formation and promoters of fibrionolysis such as t-PA, urokinase, Plamin, Streptokinase, rt-Plasminogen Activator and rstaphylokinase; antiangiogenic factors such as medroxyprogesteron, pentosan polysulphate, suramin, taxol, thalidomide, angiostatin, interferon-alpha, metalloproteinase inhibitors, platelet factor 4, somatostatin, thromobospondin; circulatory drugs such as propranolol; metabolic potentiators such as glutathione; antituberculars such as p-aminosalicylic acid, isoniazid, capreomycin sulfate, cyclosexine, ethambutol, ethionamide, pyrazinamide, rifampin or streptomycin sulphate; antivirals such as acyclovir, amantadine, azidothymidine, ribavirin or vidarabine; blood vessel dilating agents such as diltiazem, nifedipine, verapamil, erythritol tetranitrate, isosorbide dinitrate, nitroglycerin or pentaerythritol tetranitrate; antibiotics such as dapsone, chloramphenicol, neomycin, cefaclor, cefadroxil, cephalexin, cephradine, erythromycin, clindamycin, lincomycin, amoxicillin, ampicillin, bacampicillin, carbenicillin, dicloxacillin, cyclacillin, picloxacillin, hetacillin, methicillin, nafcillin, penicillin, polymyxin or tetracycline; antiinflammatories such as diflunisal, ibuprofen, indomethacin, meclefenamate, mefenamic acid, naproxen, phenylbutazone, piroxicam, tolmetin, aspirin or salicylates; antiprotozoans such as chloroquine, metronidazole, quinine or meglumine antimonate; antirheumatics such as penicillamine; narcotics such as paregoric; opiates such as codeine, morphine or opium; cardiac glycosides such as deslaneside, digitoxin, digoxin, digitalin or digitalis; neuromuscular blockers such as atracurium mesylate, gallamine triethiodide, hexafluorenium bromide, metocurine iodide, pancuronium bromide, succinylcholine chloride, tubocurarine chloride or vecuronium bromide; sedatives such as amobarbital, amobarbital sodium,

apropbarbital, butabarbital sodium, chloral hydrate, ethchlorvynol, ethinamate, flurazepam hydrochloride, glutethimide, methotrimeprazine hydrochloride, methyprylon, midazolam hydrochloride, paraldehyde, pentobarbital, secobarbital sodium, talbutal, temazepam or triazolam; local anaesthetics such as bupivacaine, chloroprocaine, etidocaine, lidocaine, mepivacaine, procaine or tetracaine; general anaesthetics such as droperidol, etomidate, fentanyl citrate with droperidol, ketamine hydrochloride, methohexital sodium or thiopental and pharmaceutically acceptable salts (e.g. acid addition salts such as the hydrochloride or hydrobromide or base salts such as sodium, calcium or magnesium salts) or derivatives (e.g. acetates) thereof. Other examples of therapeutics include genetic material such as nucleic acids, RNA, and DNA of natural or synthetic origin, including recombinant RNA and DNA. DNA encoding certain proteins may be used in the treatment of many different types of diseases. For example, tumor necrosis factor or interleukin-2 genes may be provided to treat advanced cancers; thymidine kinase genes may be provided to treat ovarian cancer or brain tumors; interleukin-2 genes may be provided to treat neuroblastoma, malignant melanoma or kidney cancer; and interleukin-4 genes may be provided to treat cancer.

Brief Summary Text - BSTX (88):

Representative examples of drugs useful in accordance with the invention include: abamectin, abundiazole, acaprazine, acabrose, acebrochol, aceburic acid, acebutolol, acecainide, acecarbromal, aceclidine, aceclofenac, acedapsone, acediasulfone, acedoben, acefluranol, acefurtiamine, acefylline clofibrol, acefylline piperazine, aceglatone, aceglutamide, aceglutamide aluminium, acemetacin, acenocoumarol, aceperone, acepromazine, acepromethazine, acequinoline, acesulfame, acetaminophen, acetaminosalol, acetanilide, acetarsone, acetazolamide, acetergamine, acetiamine, acetiromate, acetohexamide, acetohydroxamic acid, acetomeroctol, acetophenazine, acetorphine, acetosulfone, acet: ozate, acetryptine, acetylcholine chloride, acetylcolchinol, acetylcysteine, acetyldigitoxin, acetylleucine, acetylsalicyclic acid, acevaltrate, acexamic acid, acifran, acipimox, acitemate, acitretin, acivicin, aclantate, aclarubicin, aclatonium napadisilate, acodazole, aconiazide, aconitine, acoxatrine, acridorex, acrihellin, acrisorcin, acrivastine, acrocinide, acronine, actinoquinol, actodigin, acyclovir, adafenoxate, adamexine, ademetionine, adenosine phosphate, adibendan, adicillin, adimolol, adinazolam, adiphenine, aditeren, aditoprim, adrafinil, adrenalone, afloqualone, afurolol, aganodine, ajmaline, aklomide, alacepril, alafosfalin, alanine mustard, alanosine, alaproclate, alazanine triclofenate, albendazole, albendazole oxide, albuterol, albutoin, alclofenac, alcometasone dipropionate, alcloxa, alcuronium chloride, aldioxa, aldosterone, alepride, aletamine, alexidine, alfacalcidol, alfadex, alfadolone, alfaprostol, alfaxalone, alfentanil, alfuzosin, algestone acetonide, algestone acetophenide, alibendol, aliconazole, alifedrine, aliflurane, alimadol, alinidine, alipamide, alitame, alizapride, allantoin, alletorphine, allobarbital, alloclamide, allocupreide, allomethadione, allopurinol, allylestrenol, allyl isothicyanate, allylprodine, allylthiourea, almadrate sulfate, almasilate, almecillin, almestrone, alminoprofen, almitrine, almoxatone, alonacic, alonimid, aloxistatin, alozafone, alpertine, alphacetylmethadol, alphameprodine, alphamethadol, alphaprodine, alpha-vinylaziridinoethyl acetate, alpidem, alpiropride, alprazolam, alprenolol, alprostadil, alrestatin, altanserin, altapizone, alteconazole, althiazide, altrenogest, altretamine, aluminium acetate, aluminium clofibrate, aluminium subacetate, alverine, amadinone acetate, amafolone, amanozine, amantadine, amantanium bromide, amantocillin, ambasilide, ambazone, ambenonium chloride, ambenoxan, ambroxol, ambruticin, ambucaine, ambucetamide, ambuphylline, ambuside, ambutonium bromide, amcinafal, amcinafide, amcinonide, amdinocillin, amdinocillin pivoxil, amebucort, amedalin, ametantrone,

amezepine, amezinium metilsulfate, amfenac, amfepentorex, amfetaminil, amflutizole, amfonelic acid, amicarbalide, amicibone, amicloral, amicycline, amidantel, amidapsone, amidephrine, amiflamine, amifloverine, amifloxacin, amifostine, amikacin, amikhelline, amiloride, aminacrine, amindocate, amineptine, aminobenzoic acid, aminocaproic acid, aminoethyl nitrate, aminoglutethimide, aminohippuric acid, aminometradine, aminopentamide, aminophylline, aminopromazine, aminopterin, aminopyrine, aminoquinol, aminoquinuride, aminorex, aminosalicyclic acid, aminothiadiazole, aminothiazole, amiodarone, amiperone, amipheazole, amipizone, amiprilose, amiquinsin, amisometradine, amisulpride, amiterol, amithiozone, amitraz, amitriptyline, amitriptylinoxide, amixetrine, amlexanox, amlodipine, amobarbital, amodiaquine, amogastrin, amolanone, amonofide, amoproxan, amopyroquin, amorolfine, amocanate, amosulalol, amotriphene, amoxapine, amoxecaine, amoxicillin, amoxydramine camsilate, amperozide, amphecloral, amphenidone, amphetamine, amphotalide, amphotericin B, ampicillin, ampiroxicam, amprolium, ampyrimine, ampyzine, amquinate, amrinone, amsacrine, amygdalin, amylene, amylmetacresol, amyl nitrite, anagestone acetate, anagrelide, anaxirone, anazocine, anazolene, ancarolol, ancitabine, androstanediol, androstanol propionate, androstenetrione, androstenonol propionate, anethole, anguidine, anidoxime, anilamate, anileridine, aniline, anilopam, anipamil, aniracetam, anirolac, anisacril, anisindione, anisopirol, anisoylbromacrylic acid, anitrazafen, anpirtoline, ansoxetine, antafenite, antazoline, antazonite, anthelmycin, anthiolimine, anthralin, anthramycin, antienite, antimony potassium tartrate, antimony thioglycollate, antipyrine, antrafenine, apalcillin, apazone, apicycline, apomorphine, apovincamine, apraclonidine, apramycin, aprindine, aprobarbital, aprofene, aptazapine, aptocaine, arabinosylmercaptopurine, aranotin, arbaprostil, arbekacin, arclofenin, arfendazam, arginine, arginine glutamat, arildone, arnolol, aronixil, arotinolol, arpinocid, arpromidine, arsanilic acid, arsthinol, artemisinin, articaine, asaley, ascorbic acid, ascorbyl palmitate, asocainol, aspartame, aspartic acid, asperlin, aspoxicillin, astemizole, atamestane, atenolol, atipamezole, atiprosin, atolide, atracurium besilate, atromepine, atropine, atropine oxide, auranofin, aurothoiglucose, aurothioglycanide, avilamycin-A, avridine, axamozide, azabon, azabuperone, azacitodine, azaclorzine, azaconazole, azacosterol, azacyclonol, azaftozine, azaguanidine, azaloxan, azamethonium bromide, azamulin, azanator, azanidazole, azaperone; azapicyl, azaprocin, azaquinzole, azaribine, azarole, azaserine, azaspirium chloride, azastene, azastrptonigrin, azatodine, azathioprine, azauridine, azelastine, azepexole, azepindole, azetepa, azidamfenicol, azidocillin, azimexon, azintamide, azipramine, azithromycin, azlocillin, azolimine, azosemide, azotomycin, aztreonam, azumolene, bacampicillin, baclofen, bacmecillinam, balsalazide, bamaluzole, bambuterol, bamethan, bamifylline, bamipine, bamnidazole, baquiloprim, barbexaclone, barbital, barucainide, batilol, bazinaprine, becanthone, beclamide, beclobrate, beclomethasone dipropionate, beclotiamine, befiperide, befunolol, befuraline, bekanamycin, belarizine, beloxamide, bemarinone, bemegride, bemetizide, bemitradine, benactyzine, benafentrine, benanserin, benapryzine, benaxibine, benazepril, bencianol, bencisteine, benclonidine, bencyclane, bendamustine, bendazac, bendazol, benderizine, bendroflumethiazide, benethamide penicillin, benexate, benflorex, benfosformin, benfotiamine, benfurodil hemisuccinate, benhepazone, benidipine, benmoxin, benolizime, benorilate, benorterone, benoxafos, benoxaprofen, benoxinate, benperidol, benproperine, benrixate, bensalan, benserazide, bensuldazic acid, bentazepam, bentemazole, bentiamine, bentipimine, bentiromide, benurestat, benzaldehyde, benzalkonium chloride, benzaprinoxide, benzarone, benzbromarone, benzestrol, benzethidine, benzethonium chloride, benzetimide, benzilonium bromide, benzindopyrine, benziodarone, benzmalecene, benznidazole, benzobarbital, benzocaine, benzoclidine, benzoctamide, benzodepa, benzododecinium chloride, benzoic acid, benzoin, benzonatate, benzopyrronium bromide, benzoquinium chloride, benzotript, benzoxiquine, benzoxonium chloride,

benzoyl peroxide, benzoylpas, benzphetamine, benzpiperylon, benzpyrinium bromide, benzquercin, benzquinamide, benzthiazide, benztropine, benzydamine, benzylpenicillin, benzylsulfamide, beperidium iodide, bephenium naphtoate, bepiastine, bepridil, beraprost, berberine sulfate, bermastine, bermoprofen, berythromycin, besulpamide, beslunide, beta carotene, betacetylmethadol, betahistine, betaine, betameprodine, betamethadol, betamethasone, betamethasone acetate, betamethasone acibutate, betamethasone benzoate, betamethasone dipropionate, betamethasone phosphate, betamethasone valerate, betamicin, betaprodine, betaxolol, betazole, bethanechol chloride, bethanidine, betiatide, betoxycaine, bevantolol, bevonium metilsulfate, bezafibrate, bezitramide, bialamicol, bibenzonium bromide, bibrocathol, bicifadine, biclodil, biclofibrate, biclotymol, bicozamycin, bidimazium iodine, bietamiverine, bietaserpine, bifemelane, bifepramide, bifluranol, bifonazole, binedaline, binfloxacin, binfibrate, bioallethrin, bioresmethrin, biotin, bipenamol, biperiden, biphenamine, biriperone, bisacodyl, bisantrene, bis(aziridinyl) butanediol, bisbendazole, bisbentiamine, bisfenazone, bisfentidine, bismuth betanaphthol, bismuth-triglycollamate, bismuth subgallate, bismuth subsalicylate, bisorbin, bisoprolol, bisorcic, bioxatin acetate, bispyrithione magsulfex, bithionol, bithionoloxide, bitipazone, bitoterol, bitoscantate, bleomycin, bluensomycin, bofumustine, bolandiol dipropionate, bolasterone, bolazine, boldenone undecylenate, bolenol, bolmantalate, bometolol, bopindolol, bornaprine, bornaprolol, bornelone, botiacrine, boxidine, brallobarbital, brazergoline, brefonalol, bremazocine, brequinar, bretylium tosylate, brindoxime, brivundine, brobactam, broclepride, brocresine, brocrinat, brodimoprim, brofaromine, brofezil, brofoxine, brolaconazole, brolamfetamine, bromacrylide, bromadoline, bromamid, bromazepam, bromchlorenone, bromebric acid, bromerguride, brometenamine, bromfenac, bromhexine, bromindione, bromisovalum, bromociclen, bromocriptine, bromodiphenhydramine, bromofenofos, bromopride, bromoxandide, bromperidol, bromperidol decanoate, brompheniramine, bronopol, broparestrol, broperamole, bropirimine, broquinaldol, brosotamide, brosuximide, brotianide, brotizolam, brovanexine, brovincamine, broxaldine, broxaterol, broxitalamic acid, broxuridine, broxyquinoline, bruceantin, brucine, bucainide, bucetin, buciclovir, bucillamine, bucindolol, bucladesine, buclizine, buclosamide, bucloxic acid, bucolome, bucricaine, bucromarone, bucrylate, bucumolol, budesonide, budipine, budotitane, budralazine, bufenadrine, bufeniode, bufetolol, bufexamac, bufezolac, buflomedil, bufogenin, buformin, bufrolin, bufuralol, bumadizone, bumecaine, bumepidil, bumetanide, bumetrizole, bunaftine, bunamidine, bunamiodyl, bunaprolast, bunazosin, bunitrolol, bunolol, buparvaquone, bupicomide, bupivacaine, bupranolol, buprenorphine, bupropion, buquineran, buquinolate, buquiterine, buramate, burodiline, buspirone, busulfan, butabarbital, butacaine, butacetin, butaclamol, butadiazamide, butafosfan, butalamine, butalbital, butamben, butamirate, butamisole, butamoxane, butanediol cyclic sulfite, butanilicaine, butanixin, butanserin, butantrone, butaperazine, butaprost, butaverine, butedronate, buterizine, butetamate, butethamine, buthiazide, butibufen, butidrine, butikacin, butilfenin, butinazocine, butinoline, butirosin, butixirate, butobendine, butoconazole, butoprolol, butoctamide, butofilolol, butonate, butopamine, butopiprine, butoprozine, butopyrammonium iodide, butorphanol, butoxamine, butoxylate, butriptyline, butropium bromide, butylated hydroxyanisole, butylated hydroxytoluene, butylparaben, butynamine, buzepide metiodide, cabastine, cabergoline, cadralazine, cafaminol, cafedrine, caffeine, calcifediol, calcitrol, calcium citrate, calcium dobesilate, calcium glubionate, calcium gluceptate, calcium gluconate, calcium glycerophosphate, calcium hypophosphite, calcium lactate, calcium lactobionate, calcium levulinate, calcium mandelate, calcium pantothenate, calcium phosphate dibasic, calcium phophate tribasic, calcium saccharate, calcium stearate, calusterone, camazepam, cambendazole, camiverine, camostast, camphotamide, camptothecin, camylofin, canbisol, cannabinol, canrenoic acid, canrenone, cantharidine, capobenic acid, capreomycin, caproxamine, capsaicine, captamine, captodiame,

captopril, capuride, caracemide, caramiphen, carazolol, carbachol, carbadox, carbaldrate, carbamazepine, carbamide peroxide, carbantel lauryl sulfate, carbaril, carbarsone, carbaspirin calcium, carbazeran, carbazochrome, carbazachrome salicylate, carbazachrome sulfonate, carbazocine, carbeniciltin, carbenicillin indanyl, carbencillin phenyl, carbenoxolone, carbenzide, carbestrol, carbetapentane, carbidopa, carbimazole, carbinoxamine, carbiphene, carbocloral, carbocysteine, carbofenotion, carbol-fuschin, carbomycin, carboplatin, carboprost, carboprost methyl, carboquone, carbromal, carbubarb, carburazepam, carbutamide, carbuterol, carcainium chloride, carebastine, carfentanil, carfimate, carisoprodol, carmantadine, carmetizide, carmofur, carmustine, carnidazole, carnitine, carocainide, caroverine, caroxazone, carperidine, caperone, carphenazine, carpindolol, carpiramine, carprofen, carpronium chloride, carsalam, cartazolate, carteolol, carubicin, carumonam, carvedilol, carzenide, carzolamide, cathine, cathinone, cefaclor, cefadroxil, cefaloniurm, cefaloram, cefamandole, cefamandole naftate, cefaparole, cefatrizine, cefazaflur, cefazedone, cefazolin, cefbuperazone, cefcanel, cefcanel daloxate, cefedrolor, cefempidone, cefepime, cefetamet, cefetrizole, cefvitril, cefixime, cefmenoxime, cefmepidium chloride, cefmetazole, cefminox, cefodizime, cefonizid, cefoperazone, ceforanide, cefotaxime, cefotetan, cefotiam, cefoxazole, cefoxitin, cefpimizole, cefpiramide, cefpirome, cefpodoxime, cefpodoxime proxetil, cefquinome, cefrotil, cefroxadine, cefsulodin, cefsumide, ceftazidime, cefteram, ceftezole, ceftiofur, ceftiolene, ceftioxide, ceftizoxime, ceftriaxone, cefuracetime, cefuroxime, cefuraxime axetil, cefurzonam, celiprolol, cephacetrile, cephalexin, cephaloglycin, cephaloridine, cephalothin, cephapirin, cephradine, cetaben, cetamolol, cethexonium chloride, cetiedil, cetirizine, cetocycline, cetohexazine, cetophenicol, cetotiamine, cetoxime, cetraxate, chaulmosulfone, chendiol, chiniofon, chlophedianol, chloracyzine, chloral betaine, chloral hydrate, chloralose, chlorambucil, chloramine, chloramphenicol, chloramphenicol palmitate, chloramphenicol succinate, chlorazanil, chlorbenzoxamine, chlorbetamide, chlorcyclizine, chlordantoin, chlordiazepoxide, chlordimorine, chlorhexadol, chlorhexidine, chlorhexidine phosphanilate, chlorindanol, chlorisondamine chloride, chlormadinone acetate, chlormerodrin, chlormezanone, chlormidazole, chloronaphazine, chloroazodin, chlorobutanol, chlorocresol, chlorodihydroxyandrostenone, chloroethyl mesylate, 5-chloro-3'-fluoro-2'3-dideoxyuridine, chloroguanide, chlorophenothane, chloroprednisone acetate, chloroprocaine, chloropyramine, chloroquine, chloroserpidine, chlorothen, chlorothiazide, chlorotriansene, chloroxine, chloroxylenol, chlorozotocin, chlorphenesin, chlorphenesin carbamate, chlorpheniramine, chlorphenoctium amsonate, chlorphenoxamine, chlorphentermine, chlorproethazine, chlorproguanil, chlorpromazine, chlorpropamide, chlorprothixene, chlorquinaldol, chlortetracycline, chlorthalidone, chlorthenoxazine, chlorzoaxazone, chloecalciferol, cholic acid, choline chloride, choline glycerophosphate, chromocarb, chromonar, ciadox, ciamexon, cianergoline, cianidol, cianopramine, ciapilome, cicaprost, cicarperone, ciclactate, ciclafrine, ciclazindol, cicletanine, ciclomenol, ciclonicate, ciclonium bromide, ciclopirox, ciclopramine, ciclоprofen, ciclоprolol, ciclosidomine, ciclotizolam, ciclotropium bromide, cicloxilic acid, cicloxolone, cicortonide, cicrotic acid, cidoxepin, cifenline, cifostodine, ciglitazone, ciheptolane, ciladopa, cilastatine, cilazapril, cilazaprilat, cilobamine, cilofungin, cilostamide, cilostazol, ciltoprazine, cimaterol, cimemoxin, cimepanol, cimetidine, cimetropium bromide, cimoxatone, cinchonine, cinchophen, cinecromen, cinepaxadil, cinepazet, cinepazic acid, cinepazide, cinfenine, cinfenoac, cinflumide, cingestol, cinitapride, cinmetacin, cinnamaverine, cinnamedrine, cinnarizine, cinnarizine clofibrate, cinnofuradione, cincotramide, cinodine, cinolazepam, cinoquidox, cinoaxin, cinoxate, cinoxolone, cinooxopazide, cinperene, cinprazole, cinpropazide, cinromide, cintazone, cintriamide, cinperone, ciprafamide, ciprafazone, ciprefadol, ciprocinonide, ciprofibrate, ciprofloxacin, cipropride,

ciproquazone, ciprostene, ciramadol, cirazoline, cisapride, cisconazole, cismadinone, cisplatin, cistinexine, citalopram, citatepine, citenamide, citenazone, citicoline, citiolone, clamidoxic acid, clamoxyquin, clanfenur, clanobutin, clantifen, clarithromycin, clavulanic acid, clazolam, clazolimine, clazuril, clebopride, clefamide, clemastine, clemeprol, clemizole, clenbuterol, clenpirin, cletoquine, clibucaine, clidafidine, clidanac, clidinum bromide, climazolam, climbazole, climiqualine, clindamycin, clindamycin palmitate, clindamycin phosphate, clinofibrate, clinolamide, cliquinol, clioxanide, clipoxamine, cliprofen, clobazam, clobenoside, clobenzepam, clobenzorex, clobenztropine, clobetasol propionate, clobetasone butyrate, clobutinol, clobuzarit, clocanfamide, clocapramine, clociguanil, clocinizine, clocortolone acetate, clocortolone pivalate, clocoumarol, clodacaine, clodanolene, clodazon, clodoxopone, clodronic acid, clofazimine, clofenamic acid, clofenamide, clofenciclan, clofenetamine, clofenoxyde, clofenvinfos, clofeverine, clofexamide, clofezone, clofibrate, clofibric acid, clofibride, clofilium phosphate, cloflucarban, clofoctol, cloforex, clofurac, clogestone acetate, cloguanamil, clomacran, clomegestone acetate, clometacin, clometherone, clomethiazole, clometocillin, clomifenoxide, clominorex, clomiphene, clomipramine, clomocycline, clomoxir, clonazepam, clonazoline, clonidine, clonitazene, clonitrate, clonixeril, clonixin, clopamide, clopenthixol, cloperastine, cloperidone, clopidogrel, clopidol, clopimozide, clopipazan, clopirac, cloponone, cloprednol, cloprostenol, cloprothiazole, cloquinate,

US-PAT-NO: 6645974

DOCUMENT-IDENTIFIER: US 6645974 B2

TITLE: Androgen receptor modulators and methods for use thereof

DATE-ISSUED: November 11, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Hutchinson; John H.	La Jolla	CA	N/A	N/A
Breslin; Michael J.	Drexel Hill	PA	N/A	N/A
Rodan; Gideon A.	Bryn Mawr	PA	N/A	N/A
Sahoo; Soumya P.	Old Bridge	NJ	N/A	N/A
Duggan; Mark E.	Schwenksville	PA	N/A	N/A
Harada; Shun-Ichi	Ambler	PA	N/A	N/A
Schmidt; Azriel	Bryn Mawr	PA	N/A	N/A
Halczenko; Wasyl	Lansdale	PA	N/A	N/A
Towler; Dwight A.	Brentwood	MO	N/A	N/A

APPL-NO: 10/ 205634

DATE FILED: July 25, 2002

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS

The present application claims priority of U.S. provisional application Serial No. 60/308,841, filed Jul. 31, 2001.

US-CL-CURRENT: 514/284, 546/77

ABSTRACT:

Compounds of structural formula (I) as herein defined are disclosed as useful in a method for modulating the androgen receptor in a tissue selective manner in a patient in need of such modulation, as well as in a method of agonizing the androgen receptor in a patient, and in particular the method wherein the androgen receptor is antagonized in the prostate of a male patient or in the uterus of a female patient and agonized in bone and/or muscle tissue. These compounds are useful in the treatment of conditions caused by androgen deficiency or which can be ameliorated by androgen administration, including: osteoporosis, periodontal disease, bone fracture, bone damage following bone reconstructive surgery, sarcopenia, frailty, aging skin, male hypogonadism, post-menopausal symptoms in women, atherosclerosis, hypercholesterolemia, hyperlipidemia, aplastic anemia and other hematopoietic disorders, pancreatic cancer, renal cancer, arthritis and joint repair, alone or in combination with other active agents. In addition, these compounds are useful as pharmaceutical composition ingredients alone and in combination with other active agents.

25 Claims, 0 Drawing figures

Exemplary Claim Number: 1

---------- KWIC ---------

Brief Summary Text - BSTX (147):

For the treatment of atherosclerosis, hypercholesterolemia, hyperlipidemia, the compounds of structural formula I may be effectively administered in combination with one or more additional active agents. The additional active agent or agents can be lipid altering compounds such as HMG-CoA reductase inhibitors, or agents having other pharmaceutical activities, or agents that have both lipid-altering effects and other pharmaceutical activities. Examples of HMG-CoA reductase inhibitors include statins in their lactonized or dihydroxy open acid forms and pharmaceutically acceptable salts and esters thereof, including but not limited to lovastatin (see U.S. Pat. No. 4,342,767); simvastatin (see U.S. Pat. No. 4,444,784); dihydroxy open-acid simvastatin, particularly the ammonium or calcium salts thereof; pravastatin, particularly the sodium salt thereof (see U.S. Pat. No. 4,346,227); fluvastatin particularly the sodium salt thereof (see U.S. Pat. No. 5,354,772); atorvastatin, particularly the calcium salt thereof (see U.S. Pat. No. 5,273,995); cerivastatin, particularly the sodium salt thereof (see U.S. Pat. No. 5,177,080), and nisvastatin also referred to as NK-104 (see PCT international publication number WO 97/23200). Additional active agents which may be employed in combination with a compound of structural formula I include, but are not limited to, HMG-CoA synthase inhibitors; squalene epoxidase inhibitors; squalene synthetase inhibitors (also known as squalene synthase inhibitors), acyl-coenzyme A: cholesterol acyltransferase (ACAT) inhibitors including selective inhibitors of ACAT-1 or ACAT-2 as well as dual inhibitors of ACAT1 and -2; microsomal triglyceride transfer protein (MTP) inhibitors; probucol; niacin; cholesterol absorption inhibitors such as SCH-58235 also known as ezetimibe and 1-(4-fluorophenyl)-3(R)-[3(S)-(4-fluorophenyl)-3-hydroxypropyl)]-4(S)-(4-hydroxyphenyl)-2-azetidinone, which is described in U.S. Pat. No.'s 5,767,115 and 5,846,966; bile acid sequestrants; LDL (low density lipoprotein) receptor inducers; platelet aggregation inhibitors, for example glycoprotein IIb/IIIa fibrinogen receptor antagonists and aspirin; human peroxisome proliferator activated receptor gamma (PPAR.gamma.) agonists including the compounds commonly referred to as glitazones for example troglitazone, pioglitazone and rosiglitazone and, including those compounds included within the structural class known as thiazolidinediones as well as those PPAR.gamma. agonists outside the thiazolidinedione structural class; PPAR.alpha. agonists such as clofibrate, fenofibrate including micronized fenofibrate, and gemfibrozil; PPAR dual .alpha./.gamma. agonists; vitamin B.sub.6 (also known as pyridoxine) and the pharmaceutically acceptable salts thereof such as the HCl salt; vitamin B.sub.12 (also known as cyanocobalamin); folic acid or a pharmaceutically acceptable salt or ester thereof such as the sodium salt and the methylglucamine salt; anti-oxidant vitamins such as vitamin C and E and beta carotene; beta-blockers; angiotensin II antagonists such as losartan; angiotensin converting enzyme inhibitors such as enalapril and captopril; calcium channel blockers such as nifedipine and diltiazam; endothelian antagonists; agents such as LXR ligands that enhance ABC1 gene expression; bisphosphonate compounds such as alendronate sodium; and cyclooxygenase-2 inhibitors such as rofecoxib and celecoxib as well as other agents known to be useful in the treatment of these conditions.

Detailed Description Text - DETX (68):

HepG2 cells are cultured in phenol red free MEM containing 10% charcoal-treated FCS at 37C with 5% CO.sub.2. For transfection, cells are plated at 10,000 cells/well in 96 well white, clear bottom plates. Twenty four hours later, cells are co-transfected with a MMP1 promoter-luciferase reporter construct and a rhesus monkey expression construct (50: 1 ratio) using FuGENE6 transfection reagent, following the protocol recommended by manufacture. The

MMP1 promoter-luciferase reporter construct is generated by insertion of a human MMP1 promoter fragment (-179/+63) into pGL2 luciferase reporter construct (Promega) and a rhesus monkey AR expression construct is generated in a CMV-Tag2B expression vector (Stratagene). Cells are further cultured for 24 hours and then treated with ligands in the presence of 100 nM phorbol-12-myristate-13-acetate (PMA), used to increase the basal activity of MMP1 promoter. The ligands are added at this point, at a range of 1000 nM to 0.03nM, 10 dilutions, at a concentration on 10.times., 1/10th volume. (example: 10 microliters of ligand at 10.times.added to 100 microliters of media already in the well.) Cells are further cultured for additional 48 hours. Cells are then washed twice with PBS and lysed by adding 70 .mu.L of Lysis Buffer (1.times., Promega) to the wells. The luciferase activity is measured in a 96 well format using a 1450 Microbeta Jet (Perkin Elmer) luminometer. AR agonism of tissue selective androgen receptor modulators is presented as suppression of luciferase signal from the PMA-stimulated control levels EC.sub.50 and Emax values are reported. Tissue selective androgen receptor modulators of the present invention typically agonize repression typically with submicromolar EC.sub.50 values and Emax values greater than about 50%.

US-PAT-NO: 6605443

DOCUMENT-IDENTIFIER: US 6605443 B1

TITLE: Method for identifying compounds that affect Smad7 binding

DATE-ISSUED: August 12, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Nakao; Atsuhito	Chiba	N/A	N/A	JP
Heldin; Carl-Henrik	Uppsala	N/A	N/A	SE
ten Dijke; Peter	Amsterdam	N/A	N/A	NL

APPL-NO: 09/ 885722

DATE FILED: June 20, 2001

PARENT-CASE:

RELATED APPLICATIONS

This application is a divisional of application Ser. No. 09/082,092, filed May 20, 1998, now issued as U.S. Pat. No. 6,251,628, which claims priority under 35 U.S.C. .sctn.119(e) from U.S. provisional application serial No. 60/047,221, filed May 20, 1997, from U.S. provisional application serial No. 60/060,465, filed Sep. 30, 1997, from U.S. provisional application serial No. 60/075,940, filed Feb. 25, 1998, and from U.S. provisional application serial No. 60/077,033, filed Mar. 6, 1998.

US-CL-CURRENT: 435/7.8

ABSTRACT:

The invention describes nucleic acids encoding the Smad7 protein, including fragments and biologically functional variants thereof. Also included are polypeptides and fragments thereof encoded by such nucleic acids, and antibodies relating thereto. Methods and products for using such nucleic acids and polypeptides also are provided.

7 Claims, 70 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 42

---------- KWIC ---------

Detailed Description Text - DETX (72):

The assay mixture also comprises a candidate pharmacological agent. Typically, a plurality of assay mixtures are run in parallel with different agent concentrations to obtain a different response to the various concentrations. Typically, one of these concentrations serves as a negative control, i.e., at zero concentration of agent or at a concentration of agent below the limits of assay detection. Candidate agents encompass numerous

chemical classes, although typically they are organic compounds. Preferably, the candidate pharmacological agents are small organic compounds, i.e., those having a molecular weight of more than 50 yet less than about 2500, preferably less than about 1000 and, more preferably, less than about 500. Candidate agents comprise functional chemical groups necessary for structural interactions with polypeptides and/or nucleic acids, and typically include at least an amine, carbonyl, hydroxyl or carboxyl group, preferably at least two of the functional chemical groups and more preferably at least three of the functional chemical groups. The candidate agents can comprise cyclic carbon or heterocyclic structure and/or aromatic or polyaromatic structures substituted with one or more of the above-identified functional groups. Candidate agents also can be biomolecules such as peptides, saccharides, fatty acids, sterols, isoprenoids, purines, pyrimidines, derivatives or structural analogs of the above, or combinations thereof and the like. Where the agent is a nucleic acid, the agent typically is a DNA or RNA molecule, although modified nucleic acids as defined herein are also contemplated.

Detailed Description Text - DETX (119):

To investigate whether Smad7 modulates the responsiveness to TGF-.beta., the TGF-.beta.-inducible luciferase p3TPLux reporter construct, which contains the TGF-.beta.-inducible PAI-1 promoter, was transfected into Mv1Lu mink epithelial cells in the absence or presence of Smad7 cDNA. Smad7 was found to exert a dose-dependent inhibition TGF-.beta.-induce luciferase activity (FIG. 2a). Moreover, the induction of p3TPLux luciferase by a constitutively active variant of T.beta.R-I, when transfected in R-mutant cells, was also inhibited by cotransfection with Smad7, as was the response by a constitutively active variant of the structurally related type I receptor for activin (ActR-IB) (FIG. 2B). Transfection of Smad2 did not affect TGF-.beta.1-induced p3TPLux luciferase response in Mv1Lu cells (FIG. 2a). This inhibitory effect was specific as Smad7 did not inhibit the phorbol 12-myristate 13-acetate (PMA)/epidermal growth factor-induced p3TPLux luciferase response. In addition, the forskolin-mediated transcriptional induction using a cAMP-responsive-element-containing reporter construct was not affected by Smad7. These results indicate that Smad7 is a potent negative regulator of both T.beta.R-I- and ActR-IB-induced p3TPLux response.

US-PAT-NO: 6544966

DOCUMENT-IDENTIFIER: US 6544966 B1

TITLE: Agents promoting laminin production in skin cells

DATE-ISSUED: April 8, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Amano; Satoshi	Yokohama	N/A	N/A	JP
Nishiyama; Toshio	Yokohama	N/A	N/A	JP

APPL-NO: 09/ 647506

DATE FILED: September 29, 2000

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
JP	10-101910	March 31, 1998
JP	10-345677	December 4, 1998

PCT-DATA:
APPL-NO: PCT/JP99/01540
DATE-FILED: March 26, 1999
PUB-NO: WO99/49832
PUB-DATE: Oct 7, 1999
371-DATE:
102(E)-DATE:

US-CL-CURRENT: 514/78, 514/114

ABSTRACT:

Disclosed are compositions for promoting the production of laminin 5 in epidermic cells which contain a lysophospholipid having a C.sub.14-22 fatty acid residue or a preparation derived from soybeans as an active ingredient and which can be used in the fields of cosmetics and dermatology. These compositions may be used especially for the purpose of potentiating the skin.

3 Claims, 0 Drawing figures

Exemplary Claim Number: 1

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Brief Summary Text - BSTX (59):
The aforesaid powder ingredients include inorganic powders such as talc, kaolin, mica, sericite, muscovite, phlogopite, synthetic mica, lepidolite, biotite, lithia mica, vermiculite, magnesium carbonate, calcium carbonate, aluminum silicate, barium silicate, calcium silicate, magnesium silicate, strontium silicate, metallic salts of tungstic acid, magnesium, silica, zeolite, barium sulfate, burned calcium sulfate (calcined gypsum), calcium phosphate, fluorapatite, hydroxyapatite, ceramic powders, metallic soaps (e.g., zinc myristate, calcium palmitate and aluminum stearate) and boron nitride; organic powders such as powdered polyamide resins (powdered nylons), powdered

polyethylene, powdered polymethyl methacrylate, powdered polystyrene, powdered styrene-acrylic acid copolymer resin, powdered benzoguanamine resin, powdered polytetrafluoroethylene and powdered cellulose; inorganic white pigments such as titanium dioxide and zinc oxide; inorganic red pigments such as iron oxide (red oxide) and iron titanate; inorganic brown pigments such as .beta.-iron oxide; inorganic yellow pigments such as yellow iron oxide and ochre; inorganic black pigments such as black iron oxide, carbon black and lower titanium oxides; inorganic violet pigments such as manganese violet and cobalt violet; inorganic green pigments such as chromium oxide, chromium hydroxide and cobalt titanate; inorganic blue pigments such as ultramarine blue and Prussian blue; pearl pigments such as titanium oxide-coated mica, titanium oxide-coated bismuth oxychloride, titanium oxide-coated talc, colored titanium oxide-coated mica, bismuth oxychloride and pearl essence; metal powder pigments such as aluminum powder and copper powder; organic pigments such as zirconium, barium and aluminum lakes including Red No. 201, Red No. 202, Red No. 204, Red No. 205, Red No. 220, Red No. 226, Red No. 228, Red No. 405, Orange No. 203, Orange No. 204, Yellow No. 205, Yellow No. 401, Blue No. 404, Red No. 3, Red No. 104, Red No. 106, Red No. 227, Red No. 230, Red No. 401, Red No.505, Orange No. 205, Yellow No. 4, Yellow No. 5, Yellow No. 202, Yellow No. 203, Green No. 3 and Blue No. 1; natural pigments such as chlorophyll and .beta.-carotene; and coloring materials such as Titan Yellow, carthamin and Safflor Red.

Brief Summary Text - BSTX (66):

The aforesaid active ingredients include whitening agents such as arbutin, vitamin C and its derivatives, kojic acid, placental extract, glutathione and strawberry gerenium extract; anti-inflammatory agents such as glycyrrhizic acid derivatives, glycyrrhetic acid derivatives, salicylic acid derivatives, hinokitiol, zinc oxide and allantoin; activators such as royal jelly, photosensitizers, cholesterol derivatives and calf blood extract; blood circulation promoters such as nonylic acid vanillylamide, benzyl nicotinate, .beta.-butoxyethyl nicotinate, capsaicin, gingerone, cantharis tincture, ichthammol, caffeine, tannic acid, .alpha.-borneol, tocopheol nicotinate, inositol hexanicotinate, cyclandelate, cinnarizine, tolazoline, acetylcholine, verapamil, cepharanthine and .gamma.-oryzanol; antiseborrheic agents such as sulfur and thianthol; substances effective for various purposes, such as phellodedron bark extract components, coptidis rhizome extract components, lithospermum root extract components, peony root extract components, swertia herb extract components, birch extract components, sage extract components, loquat extract components, ginseng extract components, aloe extract components, mallow extract components, iris extract components, grape extract components, coix seed extract components, sponge gourd extract components, lily extract components, saffron extract components, cnidium rhizome extract components, zingiberis rhizome extract components, syoorengyo extract components, petty white-root extract components, rosemary extract components, garlic extract components, thyme extract components, capsicum extract components, citrus unshiu peel and Japanese angelica root; vitamin A compounds such as retinol and retinol acetate; vitamin B.sub.2 compounds such as riboflavin, riboflavin butyrate and flavin adenine dinucleotide; vitamin B.sub.6 compounds such as pyridoxine hydrochloride and pyridoxine dioctanoate; vitamin C compounds such as L-ascorbic acid, L-ascorbic acid dipalmitate, L-ascorbic acid 2-sulfate sodium salt, L-ascorbic acid phosphate and DL-.alpha.-tocopherol-L-ascorbic acid diphosphate dipotassium salt; pantothenic acid compounds such as calcium pantothenate, D-pantothenyl alcohol, pantothenyl ethyl ether and acetylpentothenyl ethyl ether; vitamin D compounds such as ergocalciferol and cholecarciferol; nicotinic acid compounds such as nicotinic acid, nicotinic acid amide and benzyl nicotinate; vitamin E compounds such as .alpha.-tocopherol, tocopherol acetate, DL-.alpha.-tocopherol nicotinate and DL-.alpha.-tocopherol succinate; and other vitamins such as vitamin P and

biotin.

US-PAT-NO: 6511800

DOCUMENT-IDENTIFIER: US 6511800 B1
See image for Certificate of Correction

TITLE: Methods of treating nitric oxide and cytokine mediated disorders

DATE-ISSUED: January 28, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Singh; Inderjit	Mount Pleasant	SC	N/A	N/A

APPL-NO: 09/ 579791

DATE FILED: May 25, 2000

PARENT-CASE:

This is a continuation of co-pending international application No. PCT/US98/25360 filed Nov. 25, 1998, which claims priority to U.S. Provisional Ser. No. 60/066,839, filed Nov. 25, 1997.

US-CL-CURRENT: 435/4, 435/26

ABSTRACT:

The current invention discloses novel methods for the inhibition of inducible nitric oxide synthesis (iNOS) and the production of NO. Methods of inhibiting the induction of proinflammatory cytokines are also described. Methods of treating various disease states, such as X-linked adrenoleukodystrophy, multiple sclerosis, Alzheimer's and septic shock using inhibitors of iNOS and cytokine induction are disclosed. The inhibitors include the exemplary compounds lovastatin, a sodium salt of phenylacetic acid (NaPA), FPT inhibitor II, N-acetyl cysteine (NAC), and cAMP. Methods of treating a nitric oxide or cytokine mediated disorder in a cell comprising administering a biologically effective amount of at least one induction suppressor of an inducible nitric oxide synthase or a cytokine is also described.

50 Claims, 16 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 11

---------- KWIC ---------

Brief Summary Text - BSTX (12):

Mevalonate metabolites, particularly farnesyl pyrophosphate (FPP), are involved in post-translational modification of some G-proteins, including Ras (Goldstein et al., 1990; Casey et al., 1989). The inhibition of isoprenylation of Ras proteins by inhibitors of mevalonate pathway and their membrane association and transduction of signal from Ras to Raf/MAP kinase cascade (Kikuchi et al., 1994) indicates a role of mevalonate metabolites in the

transduction of signal from receptor tyrosine kinases to Raf/MAP kinase cascade. Two enzymes that control the rate-limiting steps of the mevalonate pathway are 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, which catalyzes the formation of mevalonate from acetyl-CoA, and mevalonate pyrophosphate decarboxylase, which controls the use of mevalonate within the cell by converting 3-phospho-5-pyrophospho-mevalonate to isopentenyl pyrophosphate. Lovastatin, a potent inhibitor of HMG-CoA reductase, and sodium salt of phenylacetic acid (NaPA), an inhibitor of mevalonate pyrophosphate decarboxylase, are known to reduce the level of cellular isoprenoids (Castillo et al., 1991; Samid et al., 1994) and isoprenylated proteins (Repko and Maltese, 1989). No suppression of isoprenylated protein maturation in vitro by lovastatin treatment that produced 50% inhibition of sterol biosynthesis has been observed (Sinensky et al., 1991). The IC.sub.50 for inhibition of sterol synthesis is 10 nM, whereas the IC.sub.50 for inhibition of conversion of pro-p21.sup.ras to mature-p21.sup.ras is maximal at 2.6 .mu.M (Sinensky et al., 1991). The pharmacologically attainable concentration for NaPA, however, is 1 to 5 mM (Thibault et al., 1995). HMG-CoA reductase can also be inhibited by 5-amino 4-imidazolecarboxamide ribotide (AICAR). AICAR stimulates AMP-activated protein kinase, an enzyme that inhibits acetyl-CoA carboxylase and HMG-CoA reductase (Henin et al., 1995).

Brief Summary Text - BSTX (13):

LPS is shown to bind cell-surface receptor CD14 (Stefanova et al., 1993) and induce iNOS, probably via activation of NFk.beta. (Xie et al., 1994; Kwon et al., 1995). NFk.beta. is an ubiquitous multisubunit transcription factor that is activated in response to various stimuli including cytokines TNF-.alpha., IL-1, IL-2, IL-6, viruses, LPS, DNA damaging agents and phorbol myristate acetate (PMA) (Schreck et al., 1992). Previous studies (Law et al., 1992) demonstrating the inhibition of NF-k.beta. activation by mevinolin and 5'-methylthioadenosine indicates a role of protein farnesylation and carboxyl methylation reactions in the activation of NF-k.beta.. Identification of the binding site of NF-k.beta. in the promoter region of the iNOS gene and that the activation of NFk.beta. in LPS-induced iNOS induction establishes a role of NFk.beta. activation in the induction of iNOS (Xie et al., 1994). Although the precise mechanism of NFk.beta. activation is not known at the present time, the inhibition of activation of NFk.beta. by inhibitors of tyrosine kinase and proteases indicates a role of phosphorylation and degradation of Ik.beta. in this process (Menon et al., 1993; Henkel et al., 1993).

Detailed Description Text - DETX (403):

The following references, to the extent that they provide exemplary procedural or other details supplementary to those set forth herein, are specifically incorporated herein by reference. Abbas, A. K., et al., Cellular and Molecular Immunology, W. B. Saunders Co., Publisher, Philadelphia, Pa., 1991. Abbondanzo et al., Breast Cancer Res. Treat., 16:182(#151), 1990. Akamatsu, Ohno, Hirota, Kegoshima, Yodoi, Shigesada, "Redox regulation of the DNA binding activity in transcription factor PEBP2. The roles of two conserved cysteine residues," J. Biol. Chem. 272:14497-14500, 1997. Allred et al., Breast Cancer Res. Treat., 16:182(#149), 1990. Aruoma, O. I., et al., "The antioxidant action of N-acetyl cysteine: its reaction with hydrogen peroxide, hydroxyl radical, superoxide, and hypochlorous acid," Free Rad. Biol. Med., 6:593-597, 1989. Bagasra, Michaels, Zheng, Bobroski, Spitsin, Fu, Tawadros, Koprowski, "Activation of the inducible form of nitric oxide synthase in the brains of patients with multiple sclerosis," Proc. Natl. Acad. Sci., 92:12041-12045, 1995. Beasley and Brenner, "Role of nitric oxide in hemodialysis hypotension," Kidney Int., 42, Suppl., 38:S96-S100, 1992. Beckman et al. "Apparent hydroxyl radical production by peroxynitrite: implications for

endothelial injury from nitric oxide and superoxide," Proc. Natl. Acad. Sci. U.S.A., 87:1620-1624, 1990. Beg, Ruben, Scheinman, Haskil, Rosen, Baldwin Jr., "I kappa B interacts with the nuclear localization sequences of the subunits of NF-kappa B: a mechanism for cytoplasmic retention," Genes de Dev., 6:1899-1913, 1992. Begum and Ragolia, "cAMP counter-regulates insulin-mediated protein phosphatase-2A inactivation in rat skeletal muscle cells," J. Biol. Chem., 271:31166-31171, 1996. Berisha et al., "Nitric oxide as a mediator of oxidant lung injury due to paraquat," Proc. Natl. Acad. Sci. U.S.A., 91:744-749, 1994. Blenis, "Signal transduction via the MAP kinases: proceed at your own RSK," Proc. Natl. Acad. Sci. USA., 90:5889-5892, 1993. Bo, Dawson, Wesselingh, Mork, Choi, Kong, Hanley, Trapp, "Induction of nitric oxide synthase in demyelinating regions of multiple sclerosis brains," Ann. Neurol. 36:778-786, 1994. Bonfoco, E., et al., "Apoptosis and necrosis: two distinct events induced, respectively, by mild and intense insults with N-methyl-D-aspartate on nitric oxide/superoxide in cortical cell cultures," Proc. Natl. Acad. Sci. USA, 92:7162-7166, 1995. Boughton-Smith et al., "Nitric oxide synthase activity in ulcerative colitis and Crohns disease," Lancet 342:338-340, 1993. Bradford, M., "A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding," Anal. Biochem., 72:248-254, 1976. Bredesen, "Neural apoptosis," Ann. Neurol., 38:839-851, 1995. Brown et al., Breast Cancer Res. Treat., 16:192(#191), 1990. Brugg, Mitchel, Agid, Ruberg, "Ceramide induces apoptosis in cultured mesencephalic neurons," J. Neurochem., 66:733-739, 1996. Buisson et al., "The neuroprotective effect of a nitric oxide inhibitor in a rat model of focal cerebral ischaemia," Br. J. Pharmacol., 106:766-767, 1992. Bull et al., J. Invest. Derm., 103:435, 1994. Busse, R., et al., "Induction of nitric oxide synthase by cytokines in vascular smooth muscle cells," FEBS Lett., 275:87-90, 1990. Caira, Pacot, Bardot, Malki, Latruffe, "Transcriptional and post-transcriptional analysis of peroxisomal protein encoding genes from rat treated with an hypolipemic agent, ciprofibrate," Biochem. Pharmacol., 49:611-619, 1995. Cartier, N., J. Lopez, P. Moullier, F. Rocchiccioli, M. O. Rolland, P. Jorge, J. Mosser, J. L. Mandel, P. F. Bougneres, O. Danos, and P. Aubourg. "Retroviral-mediated gene transfer corrects very-long-chain fatty acid metabolism in adrenoleukodystrophy fibroblasts," Proc. Natl. Acad. Sci. USA. 92:1674-1678, 1995. Casey, P. J., et al., "P21 ras is modified by a farnesyl isoprenoid," Proc. Natl. Acad. Sci. USA, 86:8323-8327, 1989. Castillo, Martinez-Cayuela, Zafra, Garcia-Peregrin, "Effect of phenylalanine derivatives on the main regulatory enzymes of cholesterogenesis," Mol. Cell. Biochem., 105:21-25, 1991. Chatterjee, "Neutral sphingomyelinase," Adv. LipidRes., 26:25-47, 1993. Chen, Y and Rosazza, J. P. N., "A bacterial nitric oxide synthase from a Nocardia species. Biochem Biophys Res Commun," 203:1251-1258, 1994. Chiueh et al. "Enhanced hydroxyl radical generation by 2'-methyl analog of MPTP: suppression by clorgyline and deprenyl," Synapse 11:346-348, 1992. Contreras, M., J. Mosser, J. L. Mandel, P. Aubourg, and I. Singh. "The protein coded by the X-adrenoleukodystrophy gene is a peroxisomal integral membrane protein," FEBS Lett. 344:211-215, 1994. Contreras, M., T. K. Sengupta, F. Sheikh, P. Aubourg, and I. Singh. "Topology of ATP-binding domain of adrenoleukodystrophy gene product in peroxisomes," Arch. Biochem. Biophys. 334:369-379, 1996. Corbett and McDaniel, "Selective inhibition of inducible nitric oxide synthase by aminoguanidine," Methods Enzymol., 268:398-408, 1996. Cornell, N. W. "Rapid fractionation of cell suspensions with the use of brominated hydrocarbons," Anal. Biochem. 102:326-331, 1980. Cross, Misko, Lin, Hickey, Trotter, Tilton, "Aminoguanidine, an inhibitor of inducible nitric oxide synthase, ameliorates experimental autoimmune encephalomyelitis in SJL mice," J. Clin. Invest., 93:2684-2690, 1994. Dacrement, G., G. Cocquyt, and G. Vincent. "Measurement of very long chain fatty acids, phytanic acid and pristanic acid in plasma and cultured fibroblasts by gas chromatography," J. Inher. Metab. Dis. 18 Suppl. 1:76-83, 1995. Dawson, Dawson, London, Bredt, Snyder, "Nitric oxide mediates

glutamate neurotoxicity in primary cortical cultures," Proc. Natl. Acad. Sci USA, 88:6368-6371, 1991. Dbaio, Perry, Gamard, Platt, Poirier, Obeid, Hannun, "Cytokine response modifier A (CrmA) inhibits ceramide formation in response to tumor necrosis factor (TNF)-alpha: CrmA and Bcl-2 target distinct components in the apoptotic pathway," J. Exp. Med., 185:481490, 1997. Decrement, Cocquyt, Vincent, "Measurement of very long-chain fatty acids, phytanic and pristanic acid in plasma and cultured fibroblasts by gas chromatography," J. Inher. Metabl. Dis., 18(Suppl. 1):76-83, 1995. Devary, Gottleib, Lau, Karin, "Rapid and preferential activation of the c-jun gene during the mammalian UV response," Mol. Cell. Biol., 11:2804-2811, 1991. Devlin, J. et al., "Nitric oxide generation. A predictive parameter of acute allograft rejection," Transplantation, 58:592-595, 1994. Dignam, J. D., et al., "Accurate transcription initiation by RNA polymerase II in a soluble extract from isolated mammalian nuclei," Nucleic Acids Res., 11: 1475-1489, 1983. Dobashi, Pahan, Chahal, Singh, "Modulation of endogenous antioxidant enzymes by nitric oxide in rat C6 glial cells," J. Neurochem., 68:1896-1903, 1997. Drapier, J-C., et al., "Differentiation of murine macrophages to express nonspecific cytotoxicity for tumor cells results in L-arginine-dependent inhibition of mitochondrial iron-sulfur enzymes in the macrophages effector cells," J. Immunol., 140:2829-2838, 1988. Eberhardt et al., "Molecular cloning of the rat inducible nitric oxide synthase gene promoter," Biochem. Biophys. Res. Commun., 223:752-756, 1996. Eisieik & Leijersfam, "The inducible form of nitric oxide synthase (iNOS) in insulin-producing cells," Diabetes & Metabolism, 20:116-122, 1994. Evans et al., "Differential effects of monoclonal antibodies to tumor necrosis factor alpha and gamma interferon on induction of hepatic nitric oxide synthase in experimental gram-negative sepsis," Infec. Imm., 60:4133-4139, 1992. Feinstein, Galea, Cermak, Chugh, Lyandvert, Reis, "Nitric oxide synthase expression in glial cells: suppression by tyrosine kinase inhibitors," J. Neurochem., 62:811-814, 1994b. Feinstein, Galea, Roberts, Berquist, Wang, Reis, "Induction of nitric oxide synthase in rat C.sub.6 glial cells," J. Neurochem., 62:315-321, 1994a. Fenyk-Melody, Garrison, Brunnert, Weidner, Shen, Shelton, Mudgett, "Experimental autoimmune encephalomyelitis is exacerbated in mice lacking the NOS2 gene," J. Immunol., 160:2940-2946, 1998. Frohman, In: PCR.TM. Protocols: A Guide to Methods and Applications, Academic Press, NY, 1990. Galea, Feinstein, Reis, "Induction of calcium-independent nitric oxide synthase activity in primary rat glial cultures," Proc. Natl. Acad. Sci. USA, 89:10945-10949, 1992. Ganser, Kemer, Brown, Davisson, Kirschner, "A survey of neurological mutant mice. I. Lipid composition of myelinated tissue in known myelin mutants," Dev. Neurosci., 10:99-122, 1988. Geller, D. A., et al., "A central role for IL-1 beta in the in vitro and in vivo regulation of hepatic inducible nitric oxide synthase: IL-1 beta induces hepatic nitric oxide synthesis," J. Immunol., 155:4890-4898, 1995. Geller, Lowenstein, Shapiro, Nussler, Di Silvio, Wang, Nakayama, Simmons, Snyder, Billiar, "Molecular cloning and expression of inducible nitric oxide synthase from human hepatocytes," Proc. Natl. Acad. Sci. USA, 90:3491-3495, 1993. Geng, Y., et al., "Tyrosine kinases are involved with the expression of inducible nitric oxide synthase in human articular chondrocytes," J. Cell. Physiol., 163:545-554, 1995. Guilian, D., and T. J. Baker. "Characterization of amoebid microglia isolated from developing mammalian brain," J. Neurosci. 6:2163-2178, 1986. Goldstein, J. L., et al., "Regulation of the mevalonate pathway," Nature, 343:425-430, 1990. Goureau et al., "Lipopolysaccharide and cytokines induce a macrophage-type of nitric oxide synthase in bovine retinal pigmented epithelial cells," Biochem. Biophys. Res. Commun., 186:854-859, 1992. Graves, L. M., K. E. Bomfeildt, E. W. Raines, B. C. Potts, S. G. Macdonald, W Ross, and E. G. Krebs. 1993. "Protein kinase A antagonizes platelet-derved growth factor-induced signaling by mitogen-activated protein kinase in human arterial smooth muscle cells," Proc. Natl. Acad. Sci. USA. 90:10300-10304. Griffith, "Determination of glutathione and glutathione disulfide using glutathione reductase and

2-vinylpyridine," Anal. Biochem., 106:207-212, 1980. Guilian and Baker, "Characterization of ameboid microglia isolated from developing mammalian brain," J. Neurosci., 6:2163-2178, 1986. Haga et al., "Synthetic Alzheimer amyloid beta/A4 peptides enhance production of complement C3 component by cultured microglial cells," Brain Res., 601:88-94, 1993. Hamid et al., "Induction of nitric oxide synthase in asthma," Lancet, 342:1510-1513, 1993. Hancock, J. F., et al., "Methylation and proteolysis are essential for efficient membrane binding of prenylated p21.sup.k-ras(B)," EMBO J., 10:641-646, 1991. Hannun and Bell, "Functions of sphingolipids and sphingolipid breakdown products in cellular regulation," Science, 243:500-507, 1989. Hannun, "Functions of ceramide in coordinating cellular responses to streiss," Science, 274:1855-1858, 1996. Hannun, "The sphingomyelin cycle and the second messenger function of ceramide," J. Biol. Chem., 269:3125-3127, 1994. Hardy, S. J., A. Ferranta, A. Poulos, B. S. Robinson, D. W. Johnson, and A. W. Murray. "Effect of exogenous fatty acids with greater than 22 carbon atoms (very long chain fatty acids) on superoxide production by human neutrophils," J. Immunol. 153:1754-1761, 1994. Hashmi, M., W. Stanley, and I. Singh. "Lignoceroyl-CoASH ligase: enzyme defect in fatty acid .beta.-oxidation system in X-linked childhood adrenoleukodystrophy," FEBS Lett. 196:247-250, 1986. Henin et al., "Inhibition of fatty acid and cholesterol synthesis by stimulation of AMP-activated protein kinase," FASEB J., 9:541-546, 1995. Henkel, T., et al., "Rapid proteolysis of IkB-alpha is necessary for activation of transcription factor NF-kB," Nature, 365:182-185, 1993. Her, Lakhani, Zu, "Dual phosphorylation and autophosphorylation in mitogen-activated protein (MAP) kinase activation," Blochem. J., 296:25-31, 1993. Hibbs et al., "Evidence for cytokine-inducible nitric oxide synthesis from L-arginine in patients receiving interleukin-2 therapy," J. Clin. Invest., 89:867-877, 1992. Hooper, Bagsra, Marini, Zborek, Ohnishi, Kean, Champion, Sarker, Bobroski, Farber, Akaike, Maeda, Koprosky, "Prevention of experimental allergic encephalomyelitis by targeting nitric oxide and peroxynitrite: implications for the treatment of multiple sclerosis," Proc. Natl. Acad Sci USA., 94:2528-2533, 1997. Hooper, Spitsin, Kean, Champion, Dickson, Chaudhry, Koprowski, "Uric acid, a natural scavenger of peroxynitrite, in experimental allergic encephalomyelitis and multiple sclerosis," Proc. Natl. Acad. Sci. USA, 95:675-680, 1998. Hoshi, M., and Y. Kishimoto. "Synthesis of cerebronic acid from lignoceric acid by rat brain preparation. Some properties and distribution of the hydroxylation system," J. Biol. Chem. 248:4123-4130, 1973. Hu, S. X., et al., "Differential regulation by cytokines of human astrocyte nitric oxide production," Glia, 15:491-494, 1995. Ialenti et al., "Modulation of adjuvant arthritis by endogenous nitric oxide," Br. J. Pharmacol., 110:701-706, 1993. Issazadeh, Mustafa, Ljungdahl, Hojeberg, Dagerlind, Elde, Olsson, "Interferon-.gamma., interleukin 4 and transforming growth factor .beta. in experimental autoimmune encephalomyelitis in Lewis rats: dynamics of cellular mRNA expression in the central nervous system and lymphoid cells," J. Neurosci. Res., 40:579-590, 1995. Jaffrey, S. R. et al., "Nitric oxide: a neural messenger," Annu. Rev. Cell Dev. Biol., 11:417-440, 1995. Jayadev, Lincardic, Hannun, "Identification of arachidonic acid as a mediator of sphingomyelin hydrolysis in response to tumor necrosis factor alpha," J. Biol. Chem., 269:5757-5763, 1994. Jelinek, T., et al., "Ras-induced activation of Raf-1 is dependent on tyrosine phosphorylation," Mol. Cell. Biol., 16:1027-1034, 1996. Joshi et al., "Effect of aminoguanidine on in vivo expression of cytokines and inducible nitric oxide synthase in the lungs of endotoxemic rats," Res. Commun. Mol. Pathol. Pharmacol. Mar., 91:339-346,1996. Kantey, Feinstein, Papa, Hermi, Karasik, "Tumor necrosis factor alpha-induced phosphorylation of insulin receptor substrate-1 (IRS-1). Possible mechanism for suppression of insulin-stimulated tyrosine phosphorylation of IRS-1," J. Biol. Chem., 270:23780-23784, 1995. Kaurs & Halliwell, "Evidence for nitric oxide-mediated oxidative damage in chronic inflammation. Nitrotyrosine in serum and synovial fluid from rheumatoid

patients," FEBS Lett., 350:9-12, 1994. Kharitonov et al., "Increased nitric oxide in exhaled air of asthmatic patients," Lancet, 343:133-135, 1994. Kikuchi, A., et al., "The post-translational modification of ras p21 is important for Raf-1 activation," J. Biol. Chem., 269:20054-20059, 1994. Kilbourn et al., "N.sup.G -methyl-L-arginine inhibits tumor necrosis factor-induced hypotension: implications for the involvement of nitric oxide," Proc. Natl. Acad. Sci. U.S.A., 87:3629-3632, 1990. Klinkert, Kojima, Lesslauer, Rinner, Lassmann, Wekerle, "TNF-alpha receptor fusion protein prevents experimental auto-immune encephalomyelitis and demyelination in Lewis rats: an overview," J. Neuroimmunol., 72(2)163-168, 1997. Kolb-Bachofen et al., "Epidermal keratinocyte expression of inducible nitric oxide synthase in skin lesions of psoriasis vulgaris," Lancet, 344:139 1994. Kolesnick and Golde, "The sphingomyelin pathway in tumor necrosis factor

Other Reference Publication - OREF (11):
Casey et al., "p21ras is modified by a farnesyl isoprenoid," Proc. Natl. Acad. Sci., 86:8323-27, 1989.

Other Reference Publication - OREF (35):
McCarty et al., "Suppression of dolichol synthesis with isoprenoids and statins may potentiate the cancer-retardant efficacy of IGF-I down-regulation," Medical Hypotheses, 56:12-17, 2001.

US-PAT-NO: 6495498

DOCUMENT-IDENTIFIER: US 6495498 B2

TITLE: Detergent compositions with enhanced depositing, conditioning and softness capabilities

DATE-ISSUED: December 17, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Niemiec; Susan M.	Yardley	PA	N/A	N/A
Yeh; Hsing	Hillsborough	NJ	N/A	N/A
Gallagher; Regina	Cranbury	NJ	N/A	N/A

APPL-NO: 09/ 321074

DATE FILED: May 27, 1999

US-CL-CURRENT: 510/122, 424/401 , 424/70.12 , 424/70.19 , 510/119 , 510/121 , 510/123 , 510/129 , 510/466

ABSTRACT:

Novel "two-in-one" detergent compositions comprised of at least one water soluble silicone agent, at least one cationic conditioning agent, and a detergent. These compositions are suitable for use in shampoos, baths, and shower gels. Also described is a novel delivery system for depositing benefit agents into and onto the skin, nails, and/or hair comprised of at least one water soluble silicone and at least one cationic conditioning agent.

24 Claims, 2 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 1

---------- KWIC ---------

Detailed Description Text - DETX (84):

Examples of benefit agents suitable for treating hair loss include, but are not limited to potassium channel openers or peripheral vasodilators such as minoxidil, diazoxide, and compounds such as N*-cyano-N-(tert-pentyl)N'-3-pyridinyl-guanidine ("P-1075") as disclosed in U.S. Pat. No. 5,244,664, which is incorporated herein by reference; vitamins, such as vitamin E and vitamin C, and derivatives thereof such as vitamin E acetate and vitamin C palmitate; hormones, such as erythropoietin, prostaglandins, such as prostaglandin El and prostaglandin F2-alpha; fatty acids, such as oleic acid; diruretics such as spironolactone; heat shock proteins ("HSP"), such as HSP 27 and HSP 72; calcium channel blockers, such as verapamil HCL, nifedipine, and diltiazemamiloride; immunosuppressant drugs, such as cyclosporin and Fk-506; 5 alpha-reductase inhibitors such as finasteride; growth factors such as, EGF, IGF and FGF; transforming growth factor beta; tumor necrosis factor; non-steroidal ant-inflammatory agents such as benoxaprofen; retinoids such as tretinoin; cytokines, such as IL-6, IL-1 alpha, and IL-1 beta; cell adhension molecules such as ICAM; glucorcorticoids

such as betametasone; botanical exracts such as aloe, clove, ginseng, rehmannia, swertia, sweet orange, zanthoxylum, Serenoa repens (saw palmetto), Hypoxis rooperi, stinging nettle, pumpkin seeds, and rye pollen; other botanical exracts including sandlewool, red beet root, chrysanthemum, rosemary, burdock root and other hair growth promoter activators which are disclosed in DE 4330597 which is incorporated by reference in its entirety herein; homeopathic agents such as Kalium Phosphoricum D2, Azadirachta indica D2, and Joborandi DI; genes for cytokines, growth factors, and male-pattered baldness; antifungals such as ketoconazole and elubiol; antibiotics such as streptomycin; proteins inhibitors such as cyclohexdmide; acetazolamide; benoxaprofen; corisone; ditiazem; hexachlorobenzene; hydantoin; nifedipine; penicillamine; phenothaiazines; pinacidil; psoralens, verapamil; zidovudine; alpha-glucosylated rutin having at least one of the following rutnis: quercetin, isoquercitrin, hespeddin, naringin, and methylhesperidin, and flavonoids and transglycosidated derivatives thereof which are all disclosed in JP 7002677, which is incorporated by reference in its entirety herein; and mixtures thereof.

Detailed Description Text - DETX (90):

Examples of suitable anti-aging agents include, but are not limited to inorganic sunscreens such as titanium dioxide and zinc oxide; organic sunscreens such as octyl-methyl cinnamates and derivatives thereof; retinoids; vitamins such as vitamin E, vitamin A, vitamin C, vitamin B, and derivatives thereof such as vitamin E acetate, vitamin C palmitate, and the like; antioxidants including beta carotene, alpha hydroxy acid such as; glycolic acid, citric acid, lactic acid, malic acid, mandelic acid, ascorbic acid, alpha-hydroxbutyric acid, alpha-hydroxyisobutyric acid, alpha-hydroxyisocaproic acid, atrrolactic acid, alpha-hydroxyisovaleric acid, ethyl pyruvate, galacturonic acid, glucopehtonic acid, glucopheptono 1,4-lactone, gluconic acid, gluconolactone, glucuronic acid, glucurronolactone, glycolic acid, isopropyl pyruvate, methyl pyruvate, mucic acid, pyruvia acid, saccharic acid, saccaric acid 1,4-lactone, tartaric acid, and tatronic acid; beta hydroxy acids such as beta-hydroxybutyic acid, beta-phenyl-lactic acid, beta-phenylpyruvic acid; botanical extracts such as green tea, soy, milk thistle, algae, aloe, angelica, bitter orange, coffee, goldthread, grapefruit, hoellen, honeysuckle, Job's tears, lithospermum, mulberry, peony, puerarua, nice, safflower, and mixtures thereof.

US-PAT-NO: 6416740

DOCUMENT-IDENTIFIER: US 6416740 B1

TITLE: Acoustically active drug delivery systems

DATE-ISSUED: July 9, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Unger; Evan C.	Tucson	AZ	N/A	N/A

APPL-NO: 09/ 075343

DATE FILED: May 11, 1998

PARENT-CASE:

REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. provisional application 60/046,379, filed May 13, 1997, incorporated herein by reference in its entirety.

US-CL-CURRENT: 424/9.52, 424/450 , 424/9.5 , 424/9.51

ABSTRACT:

The present invention is directed to targeted therapeutic delivery systems comprising a gas or gaseous precursor filled microsphere wherein said gas or gaseous precursor filled microsphere comprises an oil, a surfactant, and a therapeutic compound. Methods of preparing the targeted therapeutic delivery systems are also embodied by the present invention which comprise processing a solution comprising an oil and a surfactant in the presence of a gaseous precursor, at a temperature below the gel to liquid crystalline phase transition temperature of the surfactant to form gas or gaseous precursor filled microsphere, and adding to said microspheres a therapeutic compound resulting in a targeted therapeutic delivery system, wherein said processing is selected from the group consisting of controlled agitation, controlled drying, and a combination thereof.

15 Claims, 9 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 9

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Detailed Description Text - DETX (108):

Saturated and unsaturated fatty acids which may be employed in the present stabilizing materials include molecules that preferably contain from about 12 carbon atoms to about 22 carbon atoms, in linear or branched form. Hydrocarbon groups consisting of isoprenoid units and/or prenyl groups can be used. Examples of suitable saturated fatty acids include, for example, lauric, myristic, palmitic, and stearic acids. Examples of suitable unsaturated fatty

acids include, for example, lauroleic, physeteric, myristoleic, palmitoleic, petroselinic, and oleic acids. Examples of suitable branched fatty acids include, for example, isolauric, isomyristic, isopalmitic, and isostearic acids.

Detailed Description Text - DETX (125):

Other suitable therapeutics include, antifungal agents, and bioactive agents, such as for example, antineoplastic agents, such as platinum compounds (e.g., spiroplatin, cisplatin, and carboplatin), methotrexate, adriamycin, taxol, mitomycin, ansamitocin, bleomycin, cytosine arabinoside, arabinosyl adenine, mercaptopolylysine, vincristine, busulfan, chlorambucil, melphalan (e.g., L-sarolysin (L-PAM, also known as Alkeran) and phenylalanine mustard (PAM)), mercaptopurine, mitotane, procarbazine hydrochloride, dactinomycin (actinomycin D), daunorubicin hydrochloride, doxorubicin hydrochloride, mitomycin, plicamycin (mithramycin), aminoglutethimide, estramustine phosphate sodium, flutamide, leuprolide acetate, megestrol acetate, tamoxifen citrate, testolactone, trilostane, amsacrine (m-AMSA), asparaginase (L-asparaginase) Erwina asparaginase, etoposide (VP-16), interferon .alpha.-2a, interferon .alpha.-2b, teniposide (VM-26), vinblastine sulfate (VLB), vincristine sulfate, bleomycin, bleomycin sulfate, methotrexate, adriamycin, carzelesin, and arabinosyl; blood products such as parenteral iron, hemin, hematoporphyrins and their derivatives; biological response modifiers such as muramyldipeptide, muramyltripeptide, prostaglandins, microbial cell wall components, lymphokines (e.g., bacterial endotoxin such as lipopoly-saccharide, macrophage activation factor), sub-units of bacteria (such as Mycobacteria and Corynebacteria), the synthetic dipeptide N-acetyl-muramyl-L-alanyl-D-isoglutamine; anti-fungal agents such as ketoconazole, nystatin, griseofulvin, flucytosine (5-fc), miconazole, amphotericin B, ricin, and .beta.-lactam antibiotics (e.g., sulfazecin); hormones such as growth hormone, melanocyte stimulating hormone, estradiol, beclomethasone dipropionate, betamethasone, betamethasone acetate and betamethasone sodium phosphate, vetamethasone disodium phosphate, vetamethasone sodium phosphate, cortisone acetate, dexamethasone, dexamethasone acetate, dexamethasone sodium phosphate, flunsolide, hydrocortisone, hydrocortisone acetate, hydrocortisone cypionate, hydrocortisone sodium phosphate, hydrocortisone sodium succinate, methylprednisolone, methylprednisolone acetate, methylprednisolone sodium succinate, paramethasone acetate, prednisolone, prednisolone acetate, prednisolone sodium phosphate, prednisolone tebutate, prednisone, triamcinolone, triamcinolone acetonide, triamcinolone diacetate, triamcinolone hexacetonide, fludrocortisone. acetate, progesterone, testosterone, and adrenocorticotropic hormone; vitamins such as cyanocobalamin neinoic acid, retinoids and derivatives such as retinol palmitate, .alpha.-tocopherol, naphthoquinone, cholecalciferol, folic acid, and tetrahydrofolate; peptides, such as angiostatin, manganese super oxide dismutase, tissue plasminogen activator, glutathione, insulin, dopamine, peptides with affinity for the GPIIbIIIa receptor (usually found on activated receptor platelets) such as RGD, AGD, RGE, KGD, KGE, and KQAGDV, opiate peptides (such as enkephalines and endorphins), human chorionic gonadotropin, corticotropin release factor, cholecystokinins, bradykinins, promoters of bradykinins, inhibitors of bradykinins, elastins, vasopressins, pepsins, glucagon, substance P (a pain moderation peptide), integrins, Angiotensin Converting Enzyme (ACE) inhibitors (such as captopril, enalapril, and lisinopril), adrenocorticotropic hormone, oxytocin, calcitonins, IgG, IgA, IgM, ligands for Effector Cell Protease Receptors, thrombin, streptokinase, urokinase, Protein Kinase C, interferons (such as interferon .alpha., interferon .beta., and interferon .gamma.), colony stimulating factors, granulocyte colony stimulating factors, granulocyte-macrophage colony stimulating factors, tumor necrosis factors, nerve growth factors, platelet derived growth factors, lymphotoxin, epidermal growth factors, fibroblast

growth factors, vascular endothelial cell growth factors, erythropoeitin, transforming growth factors, oncostatin M, interleukins (such as interleukin 1, interleukin 2, interleukin 3, interleukin 4, interleukin 5, interleukin 6, interleukin 7, interleukin 8, interleukin 9, interleukin 10, interleukin 11, and interleukin 12.), metalloprotein kinase ligands, and collagenases; enzymes such as alkaline phosphatase and cyclooxygenases; anti-allergic agents such as amelexanox; anti-coagulation agents such as phenprocoumon and heparin; circulatory drugs such as propranolol; metabolic potentiators such as glutathione; antituberculars such as para-aminosalicylic acid, isoniazid, capreomycin sulfate cycloserine, ethambutol hydrochloride ethionanide, pyrazinamide, rifampin, and streptomycin sulfate; antivirals such as acyclovir, amantadine azidothymidine (AZT or Zidovudine), ribavirin, amantadine, vidarabine, and vidarabine monohydrate (adenine arabinoside, ara-A); antianginals such as diltiazem, nifedipine, verapamil, erythrityl tetranitrate, isosorbide dinitrate, nitroglycerin (glyceryl trinitrate) and pentaerythritol tetranitrate; anticoagulants such as phenprocoumon, heparin; antibiotics such as dapsone, chloramphenicol, neomycin, cefaclor, cefadroxil, cephalexin, cephradine erythromycin, clindamycin, lincomycin, amoxicillin, ampicillin, bacampicillin, carbenicillin, dicloxacillin, cyclacillin, picloxacillin, hetacillin, methicillin, nafcillin, oxacillin, penicillin G, penicillin V, ticarcillin, rifampin and tetracycline; antiinflammatories such as difimisal, ibuprofen, indomethacin, meclofenamate, mefenamic acid, naproxen, oxyphenbutazone, phenylbutazone, piroxicam, sulindac, tolmetin, aspirin and salicylates; antiprotozoans such as chloroquine, hydroxychloroquine, metronidazole, quinine and meglumine antimonate; antirheumatics such as penicillamine; narcotics such as paregoric and opiates such as codeine, heroin, methadone, morphine and opium; cardiac glycosides such as deslanoside, digitoxin, digoxin, digitalin and digitalis; neuromuscular blockers such as atracurium besylate, gallamine triethiodide, hexafluorenium bromide, metocurine iodide, pancuronium bromide, succinylcholine chloride (suxamethonium chloride), tubocurarine chloride and vecuronium bromide; sedatives (hypnotics) such as amobarbital, amobarbital sodium, aprobarbital, butabarbital sodium, chloral hydrate, ethchlorvynol, ethinamate, flurazepam hydrochloride, glutethimide, methotrimeprazine hydrochloride, methyprylon, midazolam hydrochloride, paraldehyde, pentobarbital, pentobarbital sodium, phenobarbital sodium, secobarbital sodium, talbutal, temazepam and triazolam; local anesthetics such as bupivacaine hydrochloride, chloroprocaine hydrochloride, etidocaine hydrochloride, lidocaine hydrochloride, mepivacaine hydrochloride, procaine hydrochloride and tetracaine hydrochloride; general anesthetics such as droperidol, etomidate, fentanyl citrate with droperidol, ketamine hydrochloride, methohexital sodium and thiopental sodium; and radioactive particles or ions such as strontium, iodide rhenium, technetium, cobalt, and yttrium. In certain preferred embodiments, the bioactive agent is a monoclonal antibody, such as a monoclonal antibody capable of binding to melanoma antigen.

Other Reference Publication - OREF (5):
Canfield et al., "Incorporation of .beta.-Carotene into Mixed Micelles", Methods in Enzymology, 1990, 189, 418-422.

Other Reference Publication - OREF (7):
Elgorab et al., "Solubilization of .beta.-Carotene and Retinol into Aqueous Solutions of Mixed Micelles", Biochem. Biophys. Acta., 1973, 306, 58-66.

US-PAT-NO: 6331289

DOCUMENT-IDENTIFIER: US 6331289 B1

TITLE: Targeted diagnostic/therapeutic agents having more than one different vectors

DATE-ISSUED: December 18, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Klaveness; Jo	Olso	N/A	N/A	NO
Rongved; P.ang.l	Olso	N/A	N/A	NO
H.o slashed.gset; Anders	Olso	N/A	N/A	NO
Tolleshaug; Helge	Olso	N/A	N/A	NO
Cuthbertson; Alan	Olso	N/A	N/A	NO
Hoff; Lars	Olso	N/A	N/A	NO
Bryn; Klaus	Olso	N/A	N/A	NO
Hellebust; Halldis	Olso	N/A	N/A	NO
Solbakken; Magne	Olso	N/A	N/A	NO

APPL-NO: 08/ 959206

DATE FILED: October 28, 1997

PARENT-CASE:

This application claims benefit of the filing dates under 35 USC 119(e) of U.S. provisional applications 60/049,263 and 049,264 filed on Jun. 6, 1997 and 60/049,266 filed on Jun. 7, 1997.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
GB	9622366	October 28, 1996
GB	9622369	October 28, 1996
GB	9702195	February 4, 1997
GB	9708265	April 24, 1997
GB	9711837	June 6, 1997
GB	9711839	June 6, 1997

US-CL-CURRENT: 424/9.52, 424/1.21 , 424/450 , 424/9.4 , 424/9.6

ABSTRACT:

Targetable diagnostic and/or therapeutically active agents, e.g. ultrasound contrast agents, comprising a suspension in an aqueous carrier liquid of a reporter comprising gas-containing or gas-generating material, said agent being capable of forming at least two types of binding pairs with a target.

22 Claims, 1 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 1

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Detailed Description Text - DETX (88):

antineoplastic agents such as vincristine, vinblastine, vindesine, busulfan, chlorambucil, spiroplatin, cisplatin, carboplatin, methotrexate, adriamycin, mitomycin, bleomycin, cytosine arabinoside, arabinosyl adenine, mercaptopurine, mitotane, procarbazine, dactinomycin (antinomycin D), daunorubicin, doxorubicin hydrochloride, taxol, plicamycin, aminoglutethimide, estramustine, flutamide, leuprolide, megestrol acetate, tamoxifen, testolactone, trilostane, amsacrine (m-AMSA), asparaginase (L-asparaginase), etoposide, interferon a-2a and 2b, blood products such as hematoporphyrins or derivatives of the foregoing; biological response modifiers such as muramylpeptides; antifungal agents such as ketoconazole, nystatin, griseofulvin, flucytosine, miconazole or amphotericin B; hormones or hormone analogues such as growth hormone, melanocyte stimulating hormone, estradiol, beclomethasone dipropionate, betamethasone, cortisone acetate, dexamethasone, flunisolide, hydrocortisone, methylprednisolone, paramethasone acetate, prednisolone, prednisone, triamcinolone or fludrocortisone acetate; vitamins such as cyanocobalamin or retinoids; enzymes such as alkaline phosphatase or manganese superoxide dismutase; antiallergic agents such as amelexanox; inhibitors of tissue factor such as monoclonal antibodies and Fab fragments thereof, synthetic peptides, nonpeptides and compounds downregulating tissue factor expression; inhibitors of platelets such as, GPIa, GPIb and GPIIb-IIIa, ADP receptors, thrombin receptors, von Willebrand factor, prostaglandins, aspirin, ticlopidin, clopigogrel and reopro; inhibitors of coagulation protein targets such as: FIIa FVa, FVIIa, FVIIIA, FIXa, tissue factor, hepatins, hirudin, hirulog, argatroban, DEGR-rFVIIa and annexin V; inhibitors of fibrin formation and promoters of fibrionolysis such as t-PA, urokinase, Plamin, Streptokinase, rt-Plasminogen Activator and rStaphylokinase; antiangiogenic factors such as medroxyprogesteron, pentosan polysulphate, suramin, taxol, thalidomide, angiostatin, interferon-alpha, metalloproteinase inhibitors, platelet factor 4, somatostatin, thromobospondin; circulatory drugs such as propranolol; metabolic potentiators such as glutathione; antituberculars such as p-aminosalicylic acid, isoniazid, capreomycin sulfate, cyclosexine, ethambutol, ethionamide, pyrazinamide, rifampin or streptomycin sulphate; antivirals such as acyclovir, amantadine, azidothymidine, ribavirin or vidarabine; blood vessel dilating agents such as diltiazem, nifedipine, verapamil, erythritol tetranitrate, isosorbide dinitrate, nitroglycerin or pentaerythritol tetranitrate; antibiotics such as dapsone, chloramphenicol, neomycin, cefaclor, cefadroxil, cephalexin; cephradine, erythromycin, clindamycin, lincomycin, amoxicillin, ampicillin, bacampicillin, carbenicillin, dicloxacillin, cyclacillin, picloxacillin, hetacillin, methicillin, nafcillin, penicillin, polymyxin or tetracycline; antiinflammatories such as diflunisal, ibuprofen, indomethacin, meclefenamate, mefenamic acid, naproxen, phenylbutazone, piroxicam, tolmetin, aspirin or salicylates; antiprotozoans such as chloroquine, metronidazole, quinine or meglumine antimonate; antirheumatics such as penicillamine; narcotics such as paregoric; opiates such as codeine, morphine or opium; cardiac glycosides such as deslaneside, digitoxin, digoxin, digitalin or digitalis; neuromuscular blockers such as atracurium mesylate, gallamine triethiodide, hexafluorenium bromide, metocurine iodide, pancuronium bromide, succinylcholine chloride, tubocurarine chloride or vecuronium bromide; sedatives such as amobarbital, amobarbital sodium, apropbarbital, butabarbital sodium, chloral hydrate, ethchlorvynol, ethinamate, flurazepam hydrochloride, glutethimide, methotrimeprazine hydrochloride, methyprylon, midazolam hydrochloride, paraldehyde, pentobarbital, secobarbital sodium, talbutal, temazepam or triazolam; local anaesthetics such as bupivacaine, chloroprocaine, etidocaine, lidocaine, mepivacaine, procaine or tetracaine; general anaesthetics such as droperidol, etomidate, fentanyl citrate with droperidol, ketamine hydrochloride, methohexital sodium or thiopental and pharmaceutically acceptable salts (e.g. acid addition salts such as the hydrochloride or

hydrobromide or base salts such as sodium, calcium or magnesium salts) or derivatives (e.g. acetates) thereof. Other examples of therapeutics include genetic material such as nucleic acids, RNA, and DNA of natural or synthetic origin, including recombinant RNA and DNA. DNA encoding certain proteins may be used in the treatment of many different types of diseases. For example, tumor necrosis factor or interleukin-2 genes may be provided to treat advanced cancers; thymidine kinase genes may be provided to treat ovarian cancer or brain tumors; interleukin-2 genes may be provided to treat neuroblastoma, malignant melanoma or kidney cancer; and interleukin-4 genes may be provided to treat cancer.

Detailed Description Text - DETX (144):

abamectin, abundiazole, acaprazine, acabrose, acebrochol, aceburic acid, acebutolol, acecainide, acecarbromal, aceclidine, aceclofenac, acedapsone, acediasulfone, acedoben, acefluranol, acefurtiamine, acefylline clofibrol, acefylline piperazine, aceglatone, aceglutamide, aceglutamide aluminium, acemetacin, acenocoumarol, aceperone, acepromazine, aceprometazine, acequinoline, acesulfame, acetaminophen, acetaminosalol, acetanilide, acetarsone, acetazolamide, acetergamine, acetiamine, acetiromate, acetohexamide, acetohydroxamic acid, acetomeroctol, acetophenazine, acetorphine, acetosulfone, acetriozate, acetryptine, acetylcholine chloride, acetylcolchinol, acetylcysteine, acetyldigitoxin, acetylleucine, acetylsalicyclic acid, acevaltrate, acexamic acid, acifran, acipimox, acitemate, acitretin, acivicin, aclantate, aclarubicin, aclatonium napadisilate, acodazole, aconiazide, aconitine, acoxatrine, acridorex, acrihellin, acrisorcin, acrivastine, acrocinide, acronine, actinoquinol, actodigin, acyclovir, adafenoxate, adamexine, ademetionine, adenosine phosphate, adibendan, adicillin, adimolol, adinazolam, adiphenine, aditeren, aditoprim, adrafinil, adrenalone, afloqualone, afurolol, aganodine, ajmaline, aklomide, alacepril, alafosfalin, alanine mustard, alanosine, alaproclate, alazanine triclofenate, albendazole, albendazole oxide, albuterol, albutoin, alclofenac, alcometasone dipropionate, alcloxa, alcuronium chloride, aldioxa, aldosterone, alepride, aletamine, alexidine, alfacalcidol, alfadex, alfadolone, alfaprostol, alfaxalone, alfentanil, alfuzosin, algestone acetonide, algestone acetophenide, alibendol, aliconazole, alifedrine, aliflurane, alimadol, alinidine, alipamide, alitame, alizapride, allantoin, alletorphine, allobarbital, alloclamide, allocupreide, allomethadione, allopurinol, allylestrenol, allyl isothicyanate, allylprodine, allylthiourea, almadrate sulfate, almasilate, almecillin, almestrone, alminoprofen, almitrine, almoxatone, alonacic, alonimid, aloxistatin, alozafone, alpertine, alphacetylmethadol, alphameprodine, alphamethadol, alphaprodine, alpha-vinylaziridinoethyl acetate, alpidem, alpiropride, alprazolam, alprenolol, alprostadil, alrestatin, altanserin, altapizone, alteconazole, althiazide, altrenogest, altretamine, aluminium acetate, aluminium clofibrate, aluminium subacetate, alverine, amadinone acetate, amafolone, amanozine, amantadine, amantanium bromide, amantocillin, ambasilide, ambazone, ambenonium chloride, ambenoxan, ambroxol, ambruticin, ambucaine, ambucetamide, ambuphylline, ambuside, ambutonium bromide, amcinafal, amcinafide, amcinonide, amdinocillin, amdinocillin pivoxil, amebucort, amedalin, ametantrone, amezepine, amezinium metilsulfate, amfenac, amfepentorex, amfetaminil, amflutizole, amfonelic acid, amicarbalide, amicibone, amicloral, amicycline, amidantel, amidapsone, amidephrine, amiflamine, amifloverine, amifloxacin, amifostine, amikacin, amikhelline, amiloride, aminacrine, amindocate, amineptine, aminobenzoic acid, aminocaproic acid, aminoethyl nitrate, aminoglutethimide, aminohippuric acid, aminometradine, aminopentamide, aminophylline, aminopromazine, aminopterin, aminopyrine, aminoquinol, aminoquinuride, aminorex, aminosalicyclic acid, aminothiadiazole, aminothiazole, amiodarone, amiperone, amipheazole, amipizone, amiprilose,

amiquinsin, amisometradine, amisulpride, amiterol, amithiozone, amitraz, amitriptyline, amitriptylinoxide, amixetrine, amlexanox, amlodipine, amobarbital, amodiaquine, amogastrin, amolanone, amonofide, amoproxan, amopyroquin, amorolfine, amocanate, amosulalol, amotriphene, amoxapine, amoxecaine, amoxicillin, amoxydramine camsilate, amperozide, amphecloral, amphenidone, amphetamine, amphotalide, amphotericin B, ampicillin, ampiroxicam, amprolium, ampyrimine, ampyzine, amquinate, amrinone, amsacrine, amygdalin, amylene, amylmetacresol, amyl nitrite, anagestone acetate, anagrelide, anaxirone, anazocine, anazolene, ancarolol, ancitabine, androstanediol, androstanol propionate, androstenetrione, androstenonol propionate, anethole, anguidine, anidoxime, anilamate, anileridine, aniline, anilopam, anipamil, aniracetam, anirolac, anisacril, anisindione, anisopirol, anisoylbromacrylic acid, anitrazafen, anpirtoline, ansoxetine, antafenite, antazoline, antazonite, anthelmycin, anthiolimine, anthralin, anthramycin, antienite, antimony potassium tartrate, antimony thioglycollate, antipyrine, antrafenine, apalcillin, apazone, apicycline, apomorphine, apovincamine, apraclonidine, apramycin, aprindine, aprobarbital, aprofene, aptazapine, aptocaine, arabinosylmercaptopurine, aranotin, arbaprostil, arbekacin, arclofenin, arfendazam, arginine, arginine glutamat, arildone, arnolol, aronixil, arotinolol, arpinocid, arpromidine, arsanilic acid, arsthinol, artemisinin, articaine, asaley, ascorbic acid, ascorbyl palmitate, asocainol, aspartame, aspartic acid, asperlin, aspoxicillin, astemizole, atamestane, atenolol, atipamezole, atiprosin, atolide, atracurium besilate, atromepine, atropine, atropine oxide, auranofin, aurothoiglucose, aurothioglycanide, avilamycin-A, avridine, axamozide, azabon, azabuperone, azacitodine, azaclorzine, azaconazole, azacosterol, azacyclonol, azaftozine, azaguanidine, azaloxan, azamethonium bromide, azamulin, azanator, azanidazole, azaperone, azapicyl, azaprocin, azaquinzole, azaribine, azarole, azaserine, azaspirium chloride, azastene, azastrptonigrin, azatodine, azathioprine, azauridine, azelastine, azepexole, azepindole, azetepa, azidamfenicol, azidocillin, azimexon, azintamide, azipramine, azithromycin, azlocillin, azolimine, azosemide, azotomycin, aztreonam, azumolene, bacampicillin, baclofen, bacmecillinam, balsalazide, bamaluzole, bambuterol, bamethan, bamifylline, bamipine, bamnidazole, baquiloprim, barbexaclone, barbital, barucainide, batilol, bazinaprine, becanthone, beclamide, beclobrate, beclomethasone dipropionate, beclotiamine, befiperide, befunolol, befuraline, bekanamycin, belarizine, beloxamide, bemarinone, bemegride, bemetizide, bemitradine, benactyzine, benafentrine, benanserin, benapryzine, benaxibine, benazepril, bencianol, bencisteine, benclonidine, bencyclane, bendamustine, bendazac, bendazol, benderizine, bendroflumethiazide, benethamide penicillin, benexate, benflorex, benfosformin, benfotiamine, benfurodil hemisuccinate, benhepazone, benidipine, benmoxin, benolizime, benorilate, benorterone, benoxafos, benoxaprofen, benoxinate, benperidol, benproperine, benrixate, bensalan, benserazide, bensuldazic acid, bentazepam, bentemazole, bentiamine, bentipimine, bentiromide, benurestat, benzaldehyde, benzalkonium chloride, benzaprinoxide, benzarone, benzbromarone, benzestrol, benzethidine, benzethonium chloride, benzetimide, benzilonium bromide, benzindopyrine, benziodarone, benzmalecene, benznidazole, benzobarbital, benzocaine, benzoclidine, benzoctamide, benzodepa, benzododecinium chloride, benzoic acid, benzoin, benzonatate, benzopyrronium bromide, benzoquinium chloride, benzotript, benzoxiquine, benzoxonium chloride, benzoyl peroxide, benzoylpas, benzphetamine, benzpiperylon, benzpyrinium bromide, benzquercin, benzquinamide, benzthiazide, benztropine, benzydamine, benzylpenicillin, benzylsulfamide, beperidium iodide, bephenium naphtoate, bepiastine, bepridil, beraprost, berberine sulfate, bermastine, bermoprofen, berythromycin, besulpamide, beslunide, beta carotene, betacetylmethadol, betahistine, betaine, betameprodine, betamethadol, betamethasone, betamethasone acetate, betamethasone acibutate, betamethasone benzoate, betamethasone dipropionate, betamethasone phosphate, betamethasone valerate, betamicin, betaprodine, betaxolol, betazole, bethanechol chloride, bethanidine, betiatide,

betoxycaine, bevantolol, bevonium metilsulfate, bezafibrate, bezitramide, bialamicol, bibenzonium bromide, bibrocathol, bicifadine, biclodil, biclofibrate, biclotymol, bicozamycin, bidimazium iodine, bietamiverine, bietaserpine, bifemelane, bifepramide, bifluranol, bifonazole, binedaline, binfloxacin, binfibrate, bioallethrin, bioresmethrin, biotin, bipenamol, biperiden, biphenamine, biriperone, bisacodyl, bisantrene, bis(aziridinyl) butanediol, bisbendazole, bisbentiamine, bisfenazone, bisfentidine, bismuth betanaphthol, bismuth-triglycollamate, bismuth subgallate, bismuth subsalicylate, bisorbin, bisoprolol, bisorcic, bioxatin acetate, bispyrithione magsulfex, bithionol, bithionoloxide, bitipazone, bitoterol, bitoscantate, bleomycin, bluensomycin, bofumustine, bolandiol dipropionate, bolasterone, bolazine, boldenone undecylenate, bolenol, bolmantalate, bometolol, bopindolol, bornaprine, bornaprolol, bornelone, botiacrine, boxidine, brallobarbital, brazergoline, brefonalol, bremazocine, brequinar, bretylium tosylate, brindoxime, brivundine, brobactam, broclepride, brocresine, brocrinat, brodimoprim, brofaromine, brofezil, brofoxine, brolaconazole, brolamfetamine, bromacrylide, bromadoline, bromamid, bromazepam, bromchlorenone, bromebric acid, bromerguride, brometenamine, bromfenac, bromhexine, bromindione, bromisovalum, bromociclen, bromocriptine, bromodiphenhydramine, bromofenofos, bromopride, bromoxandide, bromperidol, bromperidol decanoate, brompheniramine, bronopol, broparestrol, broperamole, bropirimine, broquinaldol, brosotamide, brosuximide, brotianide, brotizolam, brovanexine, brovincamine, broxaldine, broxaterol, broxitalamic acid, broxuridine, broxyquinoline, bruceantin, brucine, bucainide, bucetin, buciclovir, bucillamine, bucindolol, bucladesine, buclizine, buclosamide, bucloxic acid, bucolome, bucricaine, bucromarone, bucrylate, bucumolol, budesonide, budipine, budotitane, budralazine, bufenadrine, bufeniode, bufetolol, bufexamac, bufezolac, buflomedil, bufogenin, buformin, bufrolin, bufuralol, bumadizone, bumecaine, bumepidil, bumetanide, bumetrizole, bunaftine, bunamidine, bunamiodyl, bunaprolast, bunazosin, bunitrolol, bunolol, buparvaquone, bupicomide, bupivacaine, bupranolol, buprenorphine, bupropion, buquineran, buquinolate, buquiterine, buramate, burodiline, buspirone, busulfan, butabarbital, butacaine, butacetin, butaclamol, butadiazamide, butafosfan, butalamine, butalbital, butamben, butamirate, butamisole, butamoxane, butanediol cyclic sulfite, butanilicaine, butanixin, butanserin, butantrone, butaperazine, butaprost, butaverine, butedronate, buterizine, butetamate, butethamine, buthiazide, butibufen, butidrine, butikacin, butilfenin, butinazocine, butinoline, butirosin, butixirate, butobendine, butoconazole, butocrolol, butoctamide, butofilolol, butonate, butopamine, butopiprine, butoprozine, butopyrammonium iodide, butorphanol, butoxamine, butoxylate, butriptyline, butropium bromide, butylated hydroxyanisole, butylated hydroxytoluene, butylparaben, butynamine, buzepide metiodide, cabastine, cabergoline, cadralazine, cafaminol, cafedrine, caffeine, calcifediol, calcitrol, calcium citrate, calcium dobesilate, calcium glubionate, calcium gluceptate, calcium gluconate, calcium glycerophosphate, calcium hypophosphite, calcium lactate, calcium lactobionate, calcium levulinate, calcium mandelate, calcium pantothenate, calcium phosphate dibasic, calcium phophate tribasic, calcium saccharate, calcium stearate, calusterone, camazepam, cambendazole, camiverine, camostast, camphotamide, camptothecin, camylofin, canbisol, cannabinol, canrenoic acid, canrenone, cantharidine, capobenic acid, capreomycin, caproxamine, capsaicine, captamine, captodiame, captopril, capuride, caracemide, caramiphen, carazolol, carbachol, carbadox, carbaldrate, carbamazepine, carbamide peroxide, carbantel lauryl sulfate, carbaril, carbarsone, carbaspirin calcium, carbazeran, carbazochrome, carbazachrome salicylate, carbazachrome sulfonate, carbazocine, carbeniciltin, carbenicillin indanyl, carbencillin phenyl, carbenoxolone, carbenzide, carbestrol, carbetapentane, carbidopa, carbimazole, carbinoxamine, carbiphene, carbocloral, carbocysteine, carbofenotion, carbol-fuschin, carbomycin, carboplatin, carboprost, carboprost methyl, carboquone, carbromal, carbubarb, carburazepam, carbutamide, carbuterol, carcainium chloride, carebastine,

carfentanil, carfimate, carisoprodol, carmantadine, carmetizide, carmofur, carmustine, carnidazole, carnitine, carocainide, caroverine, caroxazone, carperidine, caperone, carphenazine, carpindolol, carpiramine, carprofen, carpronium chloride, carsalam, cartazolate, carteolol, carubicin, carumonam, carvedilol, carzenide, carzolamide, cathine, cathinone, cefaclor, cefadroxil, cefalonium, cefaloram, cefamandole, cefamandole naftate, cefaparole, cefatrizine, cefazaflur, cefazedone, cefazolin, cefbuperazone, cefcanel, cefcanel daloxate, cefedrolor, cefempidone, cefepime, cefetamet, cefetrizole, cefvitril, cefixime, cefmenoxime, cefmepidium chloride, cefmetazole, cefminox, cefodizime, cefonizid, cefoperazone, ceforanide, cefotaxime, cefotetan, cefotiam, cefoxazole, cefoxitin, cefpimizole, cefpiramide, cefpirome, cefpodoxime, cefpodoxime proxetil, cefquinome, cefrotil, cefroxadine, cefsulodin, cefsumide, ceftazidime, cefteram, ceftezole, ceftiofur, ceftiolene, ceftioxide, ceftizoxime, ceftriaxone, cefuracetime, cefuroxime, cefuraxime axetil, cefurzonam, celiprolol, cephacetrile, cephalexin, cephaloglycin, cephaloridine, cephalothin, cephapirin, cephradine, cetaben, cetamolol, cethexonium chloride, cetiedil, cetirizine, cetocycline, cetohexazine, cetophenicol, cetotiamine, cetoxime, cetraxate, chaulmosulfone, chendiol, chiniofon, chlophedianol, chloracyzine, chloral betaine, chloral hydrate, chloralose, chlorambucil, chloramine, chloramphenicol, chloramphenicol palmitate, chloramphenicol succinate, chlorazanil, chlorbenzoxamine, chlorbetamide, chlorcyclizine, chlordantoin, chlordiazepoxide, chlordimorine, chlorhexadol, chlorhexidine, chlorhexidine phosphanilate, chlorindanol, chlorisondamine chloride, chlormadinone acetate, chlormerodrin, chlormezanone, chlormidazole, chloronaphazine, chloroazodin, chlorobutanol, chlorocresol, chlorodihydroxyandrostenone, chloroethyl mesylate, 5-chloro-3'-fluoro-2'3-dideoxyuridine, chloroguanide, chlorophenothane, chloroprednisone acetate, chloroprocaine, chloropyramine, chloroquine, chloroserpidine, chlorothen, chlorothiazide, chlorotriansene, chloroxine, chloroxylenol, chlorozotocin, chlorphenesin, chlorphenesin carbamate, chlorpheniramine, chlorphenoctium amsonate, chlorphenoxamine, chlorphentermine, chlorproethazine, chlorproguanil, chlorpromazine, chlorpropamide, chlorprothixene, chlorquinaldol, chlortetracycline, chlorthalidone, chlorthenoxazine, chlorzoaxazone, chloecalciferol, cholic acid, choline chloride, choline glycerophosphate, chromocarb, chromonar, ciadox, ciamexon, cianergoline, cianidol, cianopramine, ciapilome, cicaprost, cicarperone, ciclactate, ciclafrine, ciclazindol, cicletanine, ciclomenol, ciclonicate, ciclonium bromide, ciclopirox, ciclopramine, cicloprofen, cicloprolol, ciclosidomine, ciclotizolam, ciclotropium bromide, cicloxilic acid, cicloxolone, cicortonide, cicrotic acid, cidoxepin, cifenline, cifostodine, ciglitazone, ciheptolane, ciladopa, cilastatine, cilazapril, cilazaprilat, cilobamine, cilofungin, cilostamide, cilostazol, ciltoprazine, cimaterol, cimemoxin, cimepanol, cimetidine, cimetropium bromide, cimoxatone, cinchonine, cinchophen, cinecromen, cinepaxadil, cinepazet, cinepazic acid, cinepazide, cinfenine, cinfenoac, cinflumide, cingestol, cinitapride, cinmetacin, cinnamaverine, cinnamedrine, cinnarizine, cinnarizine clofibrate, cinnofuradione, cincotramide, cinodine, cinolazepam, cinoquidox, cinoaxin, cinoxate, cinoxolone, cinooxopazide, cinperene, cinprazole, cinpropazide, cinromide, cintazone, cintriamide, cinperone, ciprafamide, ciprafazone, ciprefadol, ciprocinonide, ciprofibrate, ciprofloxacin, cipropride, ciproquazone, ciprostene, ciramadol, cirazoline, cisapride, cisconazole, cismadinone, cisplatin, cistinexine, citalopram, citatepine, citenamide, citenazone, citicoline, citiolone, clamidoxic acid, clamoxyquin, clanfenur, clanobutin, clantifen, clarithromycin, clavulanic acid, clazolam, clazolimine, clazuril, clebopride, clefamide, clemastine, clemeprol, clemizole, clenbuterol, clenpirin, cletoquine, clibucaine, clidafidine, clidanac, clidinum bromide, climazolam, climbazole, climiqualine, clindamycin, clindamycin palmitate, clindamycin phosphate, clinofibrate, clinolamide, cliquinol, clioxanide, clipoxamine, cliprofen, clobazam, clobenoside, clobenzepam, clobenzorex,

clobenztropine, clobetasol propionate, clobetasone butyrate, clobutinol, clobuzarit, clocanfamide, clocapramine, clociguanil, clocinizine, clocortolone acetate, clocortolone pivalate, clocoumarol, clodacaine, clodanolene, clodazon, clodoxopone, clodronic acid, clofazimine, clofenamic acid, clofenamide, clofenciclan, clofenetamine, clofenoxyde, clofenvinfos, clofeverine, clofexamide, clofezone, clofibrate, clofibric acid, clofibride, clofilium phosphate, cloflucarban, clofoctol, cloforex, clofurac, clogestone acetate, cloguanamil, clomacran, clomegestone acetate, clometacin, clometherone, clomethiazole, clometocillin, clomifenoxide, clominorex, clomiphene, clomipramine, clomocycline, clomoxir, clonazepam, clonazoline, clonidine, clonitazene, clonitrate, clonixeril, clonixin, clopamide, clopenthixol, cloperastine, cloperidone, clopidogrel, clopidol, clopimozide, clopipazan, clopirac, cloponone, cloprednol, cloprostenol, cloprothiazole, cloquinate, cloquinozine, cloracetadol, cloranolol, clorazepate, clorethate, clorexolone, clorgiline, cloricromen, cloridarol, clorindanic acid,

US-PAT-NO: 6264917

DOCUMENT-IDENTIFIER: US 6264917 B1

TITLE: Targeted ultrasound contrast agents

DATE-ISSUED: July 24, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Klaveness; Jo	Oslo	N/A	N/A	NO
Rongved; P.ang.l	Oslo	N/A	N/A	NO
L.o slashed.vhaug; Dagfinn	Oslo	N/A	N/A	NO

APPL-NO: 08/ 958993

DATE FILED: October 28, 1997

PARENT-CASE:

This application claims benefit under 35 U.S.C. 119(e) of provisional applications Serial No. 60/049,264 and 60/049,265 both filed Jun. 7, 1997; and Ser. No. 60/049,268 filed Jun. 7, 1997.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
GB	9622366	October 28, 1996
GB	9622367	October 28, 1996
GB	9622368	October 28, 1996
GB	9700699	January 15, 1997
GB	9708265	April 24, 1997
GB	9711842	June 6, 1997
GB	9711846	June 6, 1997

US-CL-CURRENT: 424/9.52, 600/458

ABSTRACT:

Targetable diagnostic and/or therapeutically active agents, e.g. ultrasound contrast agents, having reporters comprising gas-filled microbubbles stabilised by monolayers of film-forming surfactants, the reporter being coupled or linked to at least one vector.

17 Claims, 2 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 2

---------- KWIC ---------

Detailed Description Text - DETX (35):

Representative and non-limiting examples of drugs useful in accordance with the invention include antineoplastic agents such as vincristine, vinblastine, vindesine, busulfan, chlorambucil, spiroplatin, cisplatin, carboplatin, methotrexate, adriamycin, mitomycin, bleomycin, cytosine arabinoside,

arabinosyl adenine, mercaptopurine, mitotane, procarbazine, dactinomycin (antinomycin D), daunorubicin, doxorubicin hydrochloride, taxol, plicamycin, aminoglutethimide, estramustine, flutamide, leuprolide, megestrol acetate, tamoxifen, testolactone, trilostane, amsacrine (m-AMSA), asparaginase (L-asparaginase), etoposide, interferon a-2a and 2b, blood products such as hematoporphyrins or derivatives of the foregoing; biological response modifiers such as muramylpeptides; antifungal agents such as ketoconazole, nystatin, griseofulvin, flucytosine, miconazole or amphotericin B; hormones or hormone analogues such as growth hormone, melanocyte stimulating hormone, estradiol, beclomethasone dipropionate, betamethasone, cortisone acetate, dexamethasone, flunisolide, hydrocortisone, methylprednisolone, paramethasone acetate, prednisolone, prednisone, triamcinolone or fludrocortisone acetate; vitamins such as cyanocobalamin or retinoids; enzymes such as alkaline phosphatase or manganese superoxide dismutase; antiallergic agents such as amelexanox; inhibitors of tissue factor such as monoclonal antibodies and Fab fragments thereof, synthetic peptides, nonpeptides and compounds downregulating tissue factor expression; inhibitors of platelets such as GPIa, GPIb and GPIIb-IIIa, ADP receptors, thrombin receptors, von Willebrand factor, prostaglandins, aspirin, ticlopidin, clopigogrel and reopro; inhibitors of coagulation protein targets such as FIIa, FVa, FVIIa, FVIIIA, FIXa, FXa, tissue factor, heparins, hirudin, hirulog, argatroban, DEGR-rFVIIa and annexin V: inhibitors of fibrin formation and promoters of fibrinolysis such as t-PA, urokinase, Plasmin, Streptokinase, rt-Plasminogen Activator and rStaphylokinase; antiangiogenic factors such as medroxyprogesteron, pentosan polysulphate, suramin, taxol, thalidomide, angiostatin, interferon-alpha, metalloproteinase inhibitors, platelet factor 4, somatostatin, thromobospondin; circulatory drugs such as propranolol; metabolic potentiators such as glutathione; antituberculars such as p-aminosalicylic acid, isoniazid, capreomycin sulfate, cyclosexine, ethambutol, ethionamide, pyrazinamide, rifampin or streptomycin sulphate; antivirals such as acyclovir, amantadine, azidothymidine, ribavirin or vidarabine; blood vessel dilating agents such as diltiazem, nifedipine, verapamil, erythritol tetranitrate, isosorbide dinitrate, nitroglycerin or pentaerythritol tetranitrate; antibiotics such as dapsone, chloramphenicol, neomycin, cefaclor, cefadroxil, cephalexin, cephradine, erythromycin, clindamycin, lincomycin, amoxicillin, ampicillin, bacampicillin, carbenicillin, dicloxacillin, cyclacillin, picloxacillin, hetacillin, methicillin, nafcillin, penicillin, polymyxin or tetracycline; antiinflammatories such as diflunisal, ibuprofen, indomethacin, meclefenamate, mefenamic acid, naproxen, phenylbutazone, piroxicam, tolmetin, aspirin or salicylates; antiprotozoans such as chloroquine, metronidazole, quinine or meglumine antimonate; antirheumatics such as penicillamine; narcotics such as paregoric; opiates such as codeine, morphine or opium; cardiac glycosides such as deslaneside, digitoxin, digoxin, digitalin or digitalis; neuromuscular blockers such as atracurium mesylate, gallamine triethiodide, hexafluorenium bromide, metocurine iodide, pancuronium bromide, succinylcholine chloride, tubocurarine chloride or vecuronium bromide; sedatives such as amobarbital, amobarbital sodium, apropbarbital, butabarbital sodium, chloral hydrate, ethchlorvynol, ethinamate, flurazepam hydrochloride, glutethimide, methotrimeprazine hydrochloride, methyprylon, midazolam hydrochloride, paraldehyde, pentobarbital, secobarbital sodium, talbutal, temazepam or triazolam; local anaesthetics such as bupivacaine, chloroprocaine, etidocaine, lidocaine, mepivacaine, procaine or tetracaine; general anaesthetics such as droperidol, etomidate, fentanyl citrate with droperidol, ketamine hydrochloride, methohexital sodium or thiopental and pharmaceutically acceptable salts (e.g. acid addition salts such as the hydrochloride or hydrobromide or base salts such as sodium, calcium or magnesium salts) or derivatives (e.g. acetates) thereof. Other examples of therapeutics include genetic material such as nucleic acids, RNA, and DNA of natural or synthetic origin, including recombinant RNA and DNA. DNA encoding certain proteins may be used in the treatment of many different types of

diseases. For example, tumor necrosis factor or interleukin-2 genes may be provided to treat advanced cancers; thymidine kinase genes may be provided to treat ovarian cancer or brain tumors; interleukin-2 genes may be provided to treat neuroblastoma, malignant melanoma or kidney cancer; and interleukin-4 genes may be provided to treat cancer.

Detailed Description Text - DETX (149):

Representative examples of drugs useful in accordance with the invention include: abamectin, abundiazole, acaprazine, acabrose, acebrochol, aceburic acid, acebutolol, acecainide, acecarbromal, aceclidine, aceclofenac, acedapsone, acediasulfone, acedoben, acefluranol, acefurtiamine, acefylline clofibrol, acefylline piperazine, aceglatone, aceglutamide, aceglutamide aluminium, acemetacin, acenocoumarol, aceperone, acepromazine, acepromethazine, acequinoline, acesulfame, acetaminophen, acetaminosalol, acetanilide, acetarsone, acetazolamide, acetergamine, acetiamine, acetiromate, acetohexamide, acetohydroxamic acid, acetomeroctol, acetophenazine, acetorphine, acetosulfone, acetriozate, acetryptine, acetylcholine chloride, acetylcolchinol, acetylcysteine, acetyldigitoxin, acetylleucine, acetylsalicyclic acid, acevaltrate, acexamic acid, acifran, acipimox, acitemate, acitretin, acivicin, aclantate, aclarubicin, aclatonium napadisilate, acodazole, aconiazide, aconitine, acoxatrine, acridorex, acrihellin, acrisorcin, acrivastine, acrocinide, acronine, actinoquinol, actodigin, acyclovir, adafenoxate, adamexine, ademetionine, adenosine phosphate, adibendan, adicillin, adimolol, adinazolam, adiphenine, aditeren, aditoprim, adrafinil, adrenalone, afloqualone, afurolol, aganodine, ajmaline, aklomide, alacepril, alafosfalin, alanine mustard, alanosine, alaproclate, alazanine triclofenate, albendazole, albendazole oxide, albuterol, albutoin, alclofenac, alcometasone dipropionate, alcloxa, alcuronium chloride, aldioxa, aldosterone, alepride, aletamine, alexidine, alfacalcidol, alfadex, alfadolone, alfaprostol, alfaxalone, alfentanil, alfuzosin, algestone acetonide, algestone acetophenide, alibendol, aliconazole, alifedrine, aliflurane, alimadol, alinidine, alipamide, alitame, alizapride, allantoin, alletorphine, allobarbital, alloclamide, allocupreide, allomethadione, allopurinol, allylestrenol, allyl isothicyanate, allylprodine, allylthiourea, almadrate sulfate, almasilate, almecillin, almestrone, alminoprofen, almitrine, almoxatone, alonacic, alonimid, aloxistatin, alozafone, alpertine, alphacetylmethadol, alphameprodine, alphamethadol, alphaprodine, alpha-vinylaziridinoethyl acetate, alpidem, alpiropride, alprazolam, alprenolol, alprostadil, alrestatin, altanserin, altapizone, alteconazole, althiazide, altrenogest, altretamine, aluminium acetate, aluminium clofibrate, aluminium subacetate, alverine, amadinone acetate, amafolone, amanozine, amantadine, amantanium bromide, amantocillin, ambasilide, ambazone, ambenonium chloride, ambenoxan, ambroxol, ambruticin, ambucaine, ambucetamide, ambuphylline, ambuside, ambutonium bromide, amcinafal, amcinafide, amcinonide, amdinocillin, amdinocillin pivoxil, amebucort, amedalin, ametantrone, amezepine, amezinium metilsulfate, amfenac, amfepentorex, amfetaminil, amflutizole, amfonelic acid, amicarbalide, amicibone, amicloral, amicycline, amidantel, amidapsone, amidephrine, amiflamine, amifloverine, amifloxacin, amifostine, amikacin, amikhelline, amiloride, aminacrine, amindocate, amineptine, aminobenzoic acid, aminocaproic acid, aminoethyl nitrate, aminoglutethimide, aminohippuric acid, aminometradine, aminopentamide, aminophylline, aminopromazine, aminopterin, aminopyrine, aminoquinol, aminoquinuride, aminorex, aminosalicyclic acid, aminothiadiazole, aminothiazole, amiodarone, amiperone, amipheazole, amipizone, amiprilose, amiquinsin, amisometradine, amisulpride, amiterol, amithiozone, amitraz, amitriptyline, amitriptylinoxide, amixetrine, amlexanox, amlodipine, amobarbital, amodiaquine, amogastrin, amolanone, amonofide, amoproxan, amopyroquin, amorolfine, amocanate, amosulalol, amotriphene, amoxapine,

amoxecaine, amoxicillin, amoxydramine camsilate, amperozide, amphecloral, amphenidone, amphetamine, amphotalide, amphotericin B, ampicillin, ampiroxicam, amprolium, ampyrimine, ampyzine, amquinate, amrinone, amsacrine, amygdalin, amylene, amylmetacresol, amyl nitrite, anagestone acetate, anagrelide, anaxirone, anazocine, anazolene, ancarolol, ancitabine, androstanediol, androstanol propionate, androstenetrione, androstenonol propionate, anethole, anguidine, anidoxime, anilamate, anileridine, aniline, anilopam, anipamil, aniracetam, anirolac, anisacril, anisindione, anisopirol, anisoylbromacrylic acid, anitrazafen, anpirtoline, ansoxetine, antafenite, antazoline, antazonite, anthelmycin, anthiolimine, anthralin, anthramycin, antienite, antimony potassium tartrate, antimony thioglycollate, antipyrine, antrafenine, apalcillin, apazone, apicycline, apomorphine, apovincamine, apraclonidine, apramycin, aprindine, aprobarbital, aprofene, aptazapine, aptocaine, arabinosylmercaptopurine, aranotin, arbaprostil, arbekacin, arclofenin, arfendazam, arginine, arginine glutamat, arildone, arnolol, aronixil, arotinolol, arpinocid, arpromidine, arsanilic acid, arsthinol, artemisinin, articaine, asaley, ascorbic acid, ascorbyl palmitate, asocainol, aspartame, aspartic acid, asperlin, aspoxicillin, astemizole, atamestane, atenolol, atipamezole, atiprosin, atolide, atracurium besilate, atromepine, atropine, atropine oxide, auranofin, aurothoiglucose, aurothioglycanide, avilamycin-A, avridine, axamozide, azabon, azabuperone, azacitodine, azaclorzine, azaconazole, azacosterol, azacyclonol, azaftozine, azaguanidine, azaloxan, azamethonium bromide, azamulin, azanator, azanidazole, azaperone, azapicyl, azaprocin, azaquinzole, azaribine, azarole, azaserine, azaspirium chloride, azastene, azastrptonigrin, azatodine, azathioprine, azauridine, azelastine, azepexole, azepindole, azetepa, azidamfenicol, azidocillin, azimexon, azintamide, azipramine, azithromycin, azlocillin, azolimine, azosemide, azotomycin, aztreonam, azumolene, bacampicillin, baclofen, bacmecillinam, balsalazide, bamaluzole, bambuterol, bamethan, bamifylline, bamipine, bamnidazole, baquiloprim, barbexaclone, barbital, barucainide, batilol, bazinaprine, becanthone, beclamide, beclobrate, beclomethasone dipropionate, beclotiamine, befiperide, befunolol, befuraline, bekanamycin, belarizine, beloxamide, bemarinone, bemegride, bemetizide, bemitradine, benactyzine, benafentrine, benanserin, benapryzine, benaxibine, benazepril, bencianol, bencisteine, benclonidine, bencyclane, bendamustine, bendazac, bendazol, benderizine, bendroflumethiazide, benethamide penicillin, benexate, benflorex, benfosformin, benfotiamine, benfurodil hemisuccinate, benhepazone, benidipine, benmoxin, benolizime, benorilate, benorterone, benoxafos, benoxaprofen, benoxinate, benperidol, benproperine, benrixate, bensalan, benserazide, bensuldazic acid, bentazepam, bentemazole, bentiamine, bentipimine, bentiromide, benurestat, benzaldehyde, benzalkonium chloride, benzaprinoxide, benzarone, benzbromarone, benzestrol, benzethidine, benzethonium chloride, benzetimide, benzilonium bromide, benzindopyrine, benziodarone, benzmalecene, benznidazole, benzobarbital, benzocaine, benzoclidine, benzoctamide, benzodepa, benzododecinium chloride, benzoic acid, benzoin, benzonatate, benzopyrronium bromide, benzoquinium chloride, benzotript, benzoxiquine, benzoxonium chloride, benzoyl peroxide, benzoylpas, benzphetamine, benzpiperylon, benzpyrinium bromide, benzquercin, benzquinamide, benzthiazide, benztropine, benzydamine, benzylpenicillin, benzylsulfamide, beperidium iodide, bephenium naphtoate, bepiastine, bepridil, beraprost, berberine sulfate, bermastine, bermoprofen, berythromycin, besulpamide, beslunide, beta carotene, betacetylmethadol, betahistine, betaine, betameprodine, betamethadol, betamethasone, betamethasone acetate, betamethasone acibutate, betamethasone benzoate, betamethasone dipropionate, betamethasone phosphate, betamethasone valerate, betamicin, betaprodine, betaxolol, betazole, bethanechol chloride, bethanidine, betiatide, betoxycaine, bevantolol, bevonium metilsulfate, bezafibrate, bezitramide, bialamicol, bibenzonium bromide, bibrocathol, bicifadine, biclodil, biclofibrate, biclotymol, bicozamycin, bidimazium iodine, bietamiverine, bietaserpine, bifemelane, bifepramide, bifluranol, bifonazole, binedaline,

binfloxacin, binfibrate, bioallethrin, bioresmethrin, biotin, bipenamol, biperiden, biphenamine, biriperone, bisacodyl, bisantrene, bis(aziridinyl) butanediol, bisbendazole, bisbentiamine, bisfenazone, bisfentidine, bismuth betanaphthol, bismuth-triglycollamate, bismuth subgallate, bismuth subsalicylate, bisorbin, bisoprolol, bisorcic, bioxatin acetate, bispyrithione magsulfex, bithionol, bithionoloxide, bitipazone, bitoterol, bitoscantate, bleomycin, bluensomycin, bofumustine, bolandiol dipropionate, bolasterone, bolazine, boldenone undecylenate, bolenol, bolmantalate, bometolol, bopindolol, bornaprine, bornaprolol, bornelone, botiacrine, boxidine, brallobarbital, brazergoline, brefonalol, bremazocine, brequinar, bretylium tosylate, brindoxime, brivundine, brobactam, broclepride, brocresine, brocrinat, brodimoprim, brofaromine, brofezil, brofoxine, brolaconazole, brolamfetamine, bromacrylide, bromadoline, bromamid, bromazepam, bromchlorenone, bromebric acid, bromerguride, brometenamine, bromfenac, bromhexine, bromindione, bromisovalum, bromociclen, bromocriptine, bromodiphenhydramine, bromofenofos, bromopride, bromoxandide, bromperidol, bromperidol decanoate, brompheniramine, bronopol, broparestrol, broperamole, bropirimine, broquinaldol, brosotamide, brosuximide, brotianide, brotizolam, brovanexine, brovincamine, broxaldine, broxaterol, broxitalamic acid, broxuridine, broxyquinoline, bruceantin, brucine, bucainide, bucetin, buciclovir, bucillamine, bucindolol, bucladesine, buclizine, buclosamide, bucloxic acid, bucolome, bucricaine, bucromarone, bucrylate, bucumolol, budesonide, budipine, budotitane, budralazine, bufenadrine, bufeniode, bufetolol, bufexamac, bufezolac, buflomedil, bufogenin, buformin, bufrolin, bufuralol, bumadizone, bumecaine, bumepidil, bumetanide, bumetrizole, bunaftine, bunamidine, bunamiodyl, bunaprolast, bunazosin, bunitrolol, bunolol, buparvaquone, bupicomide, bupivacaine, bupranolol, buprenorphine, bupropion, buquineran, buquinolate, buquiterine, buramate, burodiline, buspirone, busulfan, butabarbital, butacaine, butacetin, butaclamol, butadiazamide, butafosfan, butalamine, butalbital, butamben, butamirate, butamisole, butamoxane, butanediol cyclic sulfite, butanilicaine, butanixin, butanserin, butantrone, butaperazine, butaprost, butaverine, butedronate, buterizine, butetamate, butethamine, buthiazide, butibufen, butidrine, butikacin, butilfenin, butinazocine, butinoline, butirosin, butixirate, butobendine, butoconazole, butocrolol, butoctamide, butofilolol, butonate, butopamine, butopiprine, butoprozine, butopyrammonium iodide, butorphanol, butoxamine, butoxylate, butriptyline, butropium bromide, butylated hydroxyanisole, butylated hydroxytoluene, butylparaben, butynamine, buzepide metiodide, cabastine, cabergoline, cadralazine, cafaminol, cafedrine, caffeine, calcifediol, calcitrol, calcium citrate, calcium dobesilate, calcium glubionate, calcium gluceptate, calcium gluconate, calcium glycerophosphate, calcium hypophosphite, calcium lactate, calcium lactobionate, calcium levulinate, calcium mandelate, calcium pantothenate, calcium phosphate dibasic, calcium phophate tribasic, calcium saccharate, calcium stearate, calusterone, camazepam, cambendazole, camiverine, camostast, camphotamide, camptothecin, camylofin, canbisol, cannabinol, canrenoic acid, canrenone, cantharidine, capobenic acid, capreomycin, caproxamine, capsaicine, captamine, captodiame, captopril, capuride, caracemide, caramiphen, carazolol, carbachol, carbadox, carbaldrate, carbamazepine, carbamide peroxide, carbantel lauryl sulfate, carbaril, carbarsone, carbaspirin calcium, carbazeran, carbazochrome, carbazachrome salicylate, carbazachrome sulfonate, carbazocine, carbeniciltin, carbenicillin indanyl, carbencillin phenyl, carbenoxolone, carbenzide, carbestrol, carbetapentane, carbidopa, carbimazole, carbinoxamine, carbiphene, carbocloral, carbocysteine, carbofenotion, carbol-fuschin, carbomycin, carboplatin, carboprost, carboprost methyl, carboquone, carbromal, carbubarb, carburazepam, carbutamide, carbuterol, carcainium chloride, carebastine, carfentanil, carfimate, carisoprodol, carmantadine, carmetizide, carmofur, carmustine, carnidazole, carnitine, carocainide, caroverine, caroxazone, carperidine, caperone, carphenazine, carpindolol, carpiramine, carprofen, carpronium chloride, carsalam, cartazolate, carteolol, carubicin, carumonam,

carvedilol, carzenide, carzolamide, cathine, cathinone, cefaclor, cefadroxil, cefalonium, cefaloram, cefamandole, cefamandole naftate, cefaparole, cefatrizine, cefazaflur, cefazedone, cefazolin, cefbuperazone, cefcanel, cefcanel daloxate, cefedrolor, cefempidone, cefepime, cefetamet, cefetrizole, cefvitril, cefixime, cefmenoxime, cefmepidium chloride, cefmetazole, cefminox, cefodizime, cefonizid, cefoperazone, ceforanide, cefotaxime, cefotetan, cefotiam, cefoxazole, cefoxitin, cefpimizole, cefpiramide, cefpirome, cefpodoxime, cefpodoxime proxetil, cefquinome, cefrotil, cefroxadine, cefsulodin, cefsumide, ceftazidime, cefteram, ceftezole, ceftiofur, ceftiolene, ceftioxide, ceftizoxime, ceftriaxone, cefuracetime, cefuroxime, cefuraxime axetil, cefurzonam, celiprolol, cephacetrile, cephalexin, cephaloglycin, cephaloridine, cephalothin, cephapirin, cephradine, cetaben, cetamolol, cethexonium chloride, cetiedil, cetirizine, cetocycline, cetohexazine, cetophenicol, cetotiamine, cetoxime, cetraxate, chaulmosulfone, chendiol, chiniofon, chlophedianol, chloracyzine, chloral betaine, chloral hydrate, chloralose, chlorambucil, chloramine, chloramphenicol, chloramphenicol palmitate, chloramphenicol succinate, chlorazanil, chlorbenzoxamine, chlorbetamide, chlorcyclizine, chlordantoin, chlordiazepoxide, chlordimorine, chlorhexadol, chlorhexidine, chlorhexidine phosphanilate, chlorindanol, chlorisondamine chloride, chlormadinone acetate, chlormerodrin, chlormezanone, chlormidazole, chloronaphazine, chloroazodin, chlorobutanol, chlorocresol, chlorodihydroxyandrostenone, chloroethyl mesylate, 5-chloro-3'-fluoro-2'3-dideoxyuridine, chloroguanide, chlorophenothane, chloroprednisone acetate, chloroprocaine, chloropyramine, chloroquine, chloroserpidine, chlorothen, chlorothiazide, chlorotriansene, chloroxine, chloroxylenol, chlorozotocin, chlorphenesin, chlorphenesin carbamate, chlorpheniramine, chlorphenoctium amsonate, chlorphenoxamine, chlorphentermine, chlorproethazine, chlorproguanil, chlorpromazine, chlorpropamide, chlorprothixene, chlorquinaldol, chlortetracycline, chlorthalidone, chlorthenoxazine, chlorzoaxazone, chloecalciferol, cholic acid, choline chloride, choline glycerophosphate, chromocarb, chromonar, ciadox, ciamexon, cianergoline, cianidol, cianopramine, ciapilome, cicaprost, cicarperone, ciclactate, ciclafrine, ciclazindol, cicletanine, ciclomenol, ciclonicate, ciclonium bromide, ciclopirox, ciclopramine, cicloprofen, cicloprolol, ciclosidomine, ciclotizolam, ciclotropium bromide, cicloxilic acid, cicloxolone, cicortonide, cicrotic acid, cidoxepin, cifenline, cifostodine, ciglitazone, ciheptolane, ciladopa, cilastatine, cilazapril, cilazaprilat, cilobamine, cilofungin, cilostamide, cilostazol, ciltoprazine, cimaterol, cimemoxin, cimepanol, cimetidine, cimetropium bromide, cimoxatone, cinchonine, cinchophen, cinecromen, cinepaxadil, cinepazet, cinepazic acid, cinepazide, cinfenine, cinfenoac, cinflumide, cingestol, cinitapride, cinmetacin, cinnamaverine, cinnamedrine, cinnarizine, cinnarizine clofibrate, cinnofuradione, cincotramide, cinodine, cinolazepam, cinoquidox, cinoaxin, cinoxate, cinoxolone, cinooxopazide, cinperene, cinprazole, cinpropazide, cinromide, cintazone, cintriamide, cinperone, ciprafamide, ciprafazone, ciprefadol, ciprocinonide, ciprofibrate, ciprofloxacin, cipropride, ciproquazone, ciprostene, ciramadol, cirazoline, cisapride, cisconazole, cismadinone, cisplatin, cistinexine, citalopram, citatepine, citenamide, citenazone, citicoline, citiolone, clamidoxic acid, clamoxyquin, clanfenur, clanobutin, clantifen, clarithromycin, clavulanic acid, clazolam, clazolimine, clazuril, clebopride, clefamide, clemastine, clemeprol, clemizole, clenbuterol, clenpirin, cletoquine, clibucaine, clidafidine, clidanac, clidinum bromide, climazolam, climbazole, climiqualine, clindamycin, clindamycin palmitate, clindamycin phosphate, clinofibrate, clinolamide, cliquinol, clioxanide, clipoxamine, cliprofen, clobazam, clobenoside, clobenzepam, clobenzorex, clobenztropine, clobetasol propionate, clobetasone butyrate, clobutinol, clobuzarit, clocanfamide, clocapramine, clociguanil, clocinizine, clocortolone acetate, clocortolone pivalate, clocoumarol, clodacaine, clodanolene, clodazon, clodoxopone, clodronic acid, clofazimine, clofenamic acid, clofenamide,

clofenciclan, clofenetamine, clofenoxyde, clofenvinfos, clofeverine, clofexamide, clofezone, clofibrate, clofibric acid, clofibride, clofilium phosphate, cloflucarban, clofoctol, cloforex, clofurac, clogestone acetate, cloguanamil, clomacran, clomegestone acetate, clometacin, clometherone, clomethiazole, clometocillin, clomifenoxide, clominorex, clomiphene, clomipramine, clomocycline, clomoxir, clonazepam, clonazoline, clonidine, clonitazene, clonitrate, clonixeril, clonixin, clopamide, clopenthixol, cloperastine, cloperidone, clopidogrel, clopidol, clopimozide, clopipazan, clopirac, cloponone, cloprednol, cloprostenol, cloprothiazole, cloquinate,

US-PAT-NO: 6261537

DOCUMENT-IDENTIFIER: US 6261537 B1

TITLE: Diagnostic/therapeutic agents having microbubbles coupled to one or more vectors

DATE-ISSUED: July 17, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Klaveness; Jo	Oslo	N/A	N/A	NO
Rongved; P.ang.l	Oslo	N/A	N/A	NO
H.o slashed.gset; Anders	Oslo	N/A	N/A	NO
Tolleshaug; Helge	Oslo	N/A	N/A	NO
N.ae butted.vestad; Anne	Oslo	N/A	N/A	NO
Hellebust; Halldis	Oslo	N/A	N/A	NO
Hoff; Lars	Oslo	N/A	N/A	NO
Cuthbertson; Alan	Oslo	N/A	N/A	NO
L.o slashed.vhaug; Dagfinn	Oslo	N/A	N/A	NO
Solbakken; Magne	Oslo	N/A	N/A	NO

APPL-NO: 08/ 960054

DATE FILED: October 29, 1997

PARENT-CASE:

Applicants hereby claim benefit under 35 U.S.C. .sctn.119(e) of provisional application No. 60/049,264 filed Jun. 7, 1997, provisional application No. 60/049,265 filed Jun. 7, 1997 and provisional application No. 60/049,268 also filed Jun. 7, 1997 and is a continuation-in-part of application Ser. No. 08/958,993, filed Oct. 28, 1997, pending.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
GB	9622366	October 28, 1996
GB	9622367	October 28, 1996
GB	9622368	October 28, 1996
GB	9700699	January 15, 1997
GB	9708265	April 24, 1997
GB	9711842	June 6, 1997
GB	9711846	June 6, 1997

US-CL-CURRENT: 424/9.52, 424/1.29 , 424/489 , 424/9.32 , 424/9.4 , 424/9.6

ABSTRACT:

Targetable diagnostic and/or therapeutically active agents, e.g. ultrasound contrast agents, having reporters comprising gas-filled microbubbles stabilised by monolayers of film-forming surfactants, the reporter being coupled or linked to at least one vector.

22 Claims, 2 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 2

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Drawing Description Text - DRTX (38):

Representative and non-limiting examples of drugs useful in accordance with the invention include antineoplastic agents such as vincristine, vinblastine, vindesine, busulfan, chlorambucil, spiroplatin, cisplatin, carboplatin, methotrexate, adriamycin, mitomycin, bleomycin, cytosine arabinoside, arabinosyl adenine, mercaptopurine, mitotane, procarbazine, dactinomycin (antinomycin D), daunorubicin, doxorubicin hydrochloride, taxol, plicamycin, aminoglutethimide, estramustine, flutamide, leuprolide, megestrol acetate, tamoxifen, testolactone, trilostane, amsacrine (m-AMSA), asparaginase (L-asparaginase), etoposide, interferon a-2a and 2b, blood products such as hematoporphyrins or derivatives of the foregoing; biological response modifiers such as muramylpeptides; antifungal agents such as ketoconazole, nystatin, griseofulvin, flucytosine, miconazole or amphotericin B; hormones or hormone analogues such as growth hormone, melanocyte stimulating hormone, estradiol, beclomethasone dipropionate, betamethasone, cortisone acetate, dexamethasone, flunisolide, hydrocortisone, methylprednisolone, paramethasone acetate, prednisolone, prednisone, triamcinolone or fludrocortisone acetate; vitamins such as cyanocobalamin or retinoids; enzymes such as alkaline phosphatase or manganese superoxide dismutase; antiallergic agents such as amelexanox; inhibitors of tissue factor such as monoclonal antibodies and Fab fragments thereof, synthetic peptides, nonpeptides and compounds downregulating tissue factor expression; inhibitors of platelets such as GPIa, GPIb and GPIIb-IIIa, ADP receptors, thrombin receptors, von Willebrand factor, prostaglandins, aspirin, ticlopidin, clopigogrel and reopro; inhibitors of coagulation protein targets such as FIIa, FVa, FVIIa, FVIIIA, FIXa, FXa, tissue factor, heparins, hirudin, hirulog, argatroban, DEGR-rFVIIa and annexin V: inhibitors of fibrin formation and promoters of fibrinolysis such as t-PA, urokinase, Plasmin, Streptokinase, rt-Plasminogen Activator and rStaphylokinase; antiangiogenic factors such as medroxyprogesteron, pentosan polysulphate, suramin, taxol, thalidomide, angiostatin, interferon-alpha, metalloproteinase inhibitors, platelet factor 4, somatostatin, thromobospondin; circulatory drugs such as propranolol; metabolic potentiators such as glutathione; antituberculars such as p-aminosalicylic acid, isoniazid, capreomycin sulfate, cyclosexine, ethambutol, ethionamide, pyrazinamide, rifampin or streptomycin sulphate; antivirals such as acyclovir, amantadine, azidothymidine, ribavirin or vidarabine; blood vessel dilating agents such as diltiazem, nifedipine, verapamil, erythritol tetranitrate, isosorbide dinitrate, nitroglycerin or pentaerythritol tetranitrate; antibiotics such as dapsone, chloramphenicol, neomycin, cefaclor, cefadroxil, cephalexin, cephradine, erythromycin, clindamycin, lincomycin, amoxicillin, ampicillin, bacampicillin, carbenicillin, dicloxacillin, cyclacillin, picloxacillin, hetacillin, methicillin, nafcillin, penicillin, polymyxin or tetracycline; antiinflammatories such as diflunisal, ibuprofen, indomethacin, meclefenamate, mefenamic acid, naproxen, phenylbutazone, piroxicam, tolmetin, aspirin or salicylates; antiprotozoans such as chloroquine, metronidazole, quinine or meglumine antimonate; antirheumatics such as penicillamine; narcotics such as paregoric; opiates such as codeine, morphine or opium; cardiac glycosides such as deslaneside, digitoxin, digoxin, digitalin or digitalis; neuromuscular blockers such as atracurium mesylate, gallamine triethiodide, hexafluorenium bromide, metocurine iodide, pancuronium bromide, succinylcholine chloride, tubocurarine chloride or vecuronium bromide; sedatives such as amobarbital, amobarbital sodium, apropbarbital, butabarbital sodium, chloral hydrate, ethchlorvynol, ethinamate, flurazepam hydrochloride, glutethimide, methotrimeprazine hydrochloride, methyprylon, midazolam hydrochloride, paraldehyde, pentobarbital, secobarbital

sodium, talbutal, temazepam or triazolam; local anaesthetics such as bupivacaine, chloroprocaine, etidocaine, lidocaine, mepivacaine, procaine or tetracaine; general anaesthetics such as droperidol, etomidate, fentanyl citrate with droperidol, ketamine hydrochloride, methohexital sodium or thiopental and pharmaceutically acceptable salts (e.g. acid addition salts such as the hydrochloride or hydrobromide or base salts such as sodium, calcium or magnesium salts) or derivatives (e.g. acetates) thereof. Other examples of therapeutics include genetic material such as nucleic acids, RNA, and DNA of natural or synthetic origin, including recombinant RNA and DNA. DNA encoding certain proteins may be used in the treatment of many different types of diseases. For example, tumor necrosis factor or interleukin-2 genes may be provided to treat advanced cancers; thymidine kinase genes may be provided to treat ovarian cancer or brain tumors; interleukin-2 genes may be provided to treat neuroblastoma, malignant melanoma or kidney cancer; and interleukin-4 genes may be provided to treat cancer.

Drawing Description Text - DRTX (198):

Representative examples of drugs useful in accordance with the invention include: abamectin, abundiazole, acaprazine, acabrose, acebrochol, aceburic acid, acebutolol, acecainide, acecarbromal, aceclidine, aceclofenac, acedapsone, acediasulfone, acedoben, acefluranol, acefurtiamine, acefylline clofibrol, acefylline piperazine, aceglatone, aceglutamide, aceglutamide aluminium, acemetacin, acenocoumarol, aceperone, acepromazine, acepromethazine, acequinoline, acesulfame, acetaminophen, acetaminosalol, acetanilide, acetarsone, acetazolamide, acetergamine, acetiamine, acetiromate, acetohexamide, acetohydroxamic acid, acetomeroctol, acetophenazine, acetorphine, acetosulfone, acetriozate, acetryptine, acetylcholine chloride, acetylcolchinol, acetylcysteine, acetyldigitoxin, acetylleucine, acetylsalicyclic acid, acevaltrate, acexamic acid, acifran, acipimox, acitemate, acitretin, acivicin, aclantate, aclarubicin, aclatonium napadisilate, acodazole, aconiazide, aconitine, acoxatrine, acridorex, acrihellin, acrisorcin, acrivastine, acrocinide, acronine, actinoquinol, actodigin, acyclovir, adafenoxate, adamexine, ademetionine, adenosine phosphate, adibendan, adicillin, adimolol, adinazolam, adiphenine, aditeren, aditoprim, adrafinil, adrenalone, afloqualone, afurolol, aganodine, ajmaline, aklomide, alacepril, alafosfalin, alanine mustard, alanosine, alaproclate, alazanine triclofenate, albendazole, albendazole oxide, albuterol, albutoin, alclofenac, alcometasone dipropionate, alcloxa, alcuronium chloride, aldioxa, aldosterone, alepride, aletamine, alexidine, alfacalcidol, alfadex, alfadolone, alfaprostol, alfaxalone, alfentanil, alfuzosin, algestone acetonide, algestone acetophenide, alibendol, aliconazole, alifedrine, aliflurane, alimadol, alinidine, alipamide, alitame, alizapride, allantoin, alletorphine, allobarbital, alloclamide, allocupreide, allomethadione, allopurinol, allylestrenol, allyl isothicyanate, allylprodine, allylthiourea, almadrate sulfate, almasilate, almecillin, almestrone, alminoprofen, almitrine, almoxatone, alonacic, alonimid, aloxistatin, alozafone, alpertine, alphacetylmethadol, alphameprodine, alphamethadol, alphaprodine, alphavinylaziridinoethyl acetate, alpidem, alpiropride, alprazolam, alprenolol, alprostadil, alrestatin, altanserin, altapizone, alteconazole, althiazide, altrenogest, altretamine, aluminium acetate, aluminium clofibrate, aluminium subacetate, alverine, amadinone acetate, amafolone, amanozine, amantadine, amantanium bromide, amantocillin, ambasilide, ambazone, ambenonium chloride, ambenoxan, ambroxol, ambruticin, ambucaine, ambucetamide, ambuphylline, ambuside, ambutonium bromide, amcinafal, amcinafide, amcinonide, amdinocillin, amdinocillin pivoxil, amebucort, amedalin, ametantrone, amezepine, amezinium metilsulfate, amfenac, amfepentorex, amfetaminil, amflutizole, amfonelic acid, amicarbalide, amicibone, amicloral, amicycline, amidantel, amidapsone, amidephrine, amiflamine, amifloverine, amifloxacin, amifostine, amikacin,

amikhelline, amiloride, aminacrine, amindocate, amineptine, aminobenzoic acid, aminocaproic acid, aminoethyl nitrate, aminoglutethimide, aminohippuric acid, aminometradine, aminopentamide, aminophylline, aminopromazine, aminopterin, aminopyrine, aminoquinol, aminoquinuride, aminorex, aminosalicyclic acid, aminothiadiazole, aminothiazole, amiodarone, amiperone, amipheazole, amipizone, amiprilose, amiquinsin, amisometradine, amisulpride, amiterol, amithiozone, amitraz, amitriptyline, amitriptylinoxide, amixetrine, amlexanox, amlodipine, amobarbital, amodiaquine, amogastrin, amolanone, amonofide, amoproxan, amopyroquin, amorolfine, amocanate, amosulalol, amotriphene, amoxapine, amoxecaine, amoxicillin, amoxydramine camsilate, amperozide, amphecloral, amphenidone, amphetamine, amphotalide, amphotericin B, ampicillin, ampiroxicam, amprolium, ampyrimine, ampyzine, amquinate, amrinone, amsacrine, amygdalin, amylene, amylmetacresol, amyl nitrite, anagestone acetate, anagrelide, anaxirone, anazocine, anazolene, ancarolol, ancitabine, androstanediol, androstanol propionate, androstenetrione, androstenonol propionate, anethole, anguidine, anidoxime, anilamate, anileridine, aniline, anilopam, anipamil, aniracetam, anirolac, anisacril, anisindione, anisopirol, anisoylbromacrylic acid, anitrazafen, anpirtoline, ansoxetine, antafenite, antazoline, antazonite, anthelmycin, anthiolimine, anthralin, anthramycin, antienite, antimony potassium tartrate, antimony thioglycollate, antipyrine, antrafenine, apalcillin, apazone, apicycline, apomorphine, apovincamine, apraclonidine, apramycin, aprindine, aprobarbital, aprofene, aptazapine, aptocaine, arabinosylmercaptopurine, aranotin, arbaprostil, arbekacin, arclofenin, arfendazam, arginine, arginine glutamat, arildone, arnolol, aronixil, arotinolol, arpinocid, arpromidine, arsanilic acid, arsthinol, artemisinin, articaine, asaley, ascorbic acid, ascorbyl palmitate, asocainol, aspartame, aspartic acid, asperlin, aspoxicillin, astemizole, atamestane, atenolol, atipamezole, atiprosin, atolide, atracurium besilate, atromepine, atropine, atropine oxide, auranofin, aurothoiglucose, aurothioglycanide, avilamycin-A, avridine, axamozide, azabon, azabuperone, azacitodine, azaclorzine, azaconazole, azacosterol, azacyclonol, azaftozine, azaguanidine, azaloxan, azamethonium bromide, azamulin, azanator, azanidazole, azaperone, azapicyl, azaprocin, azaquinzole, azaribine, azarole, azaserine, azaspirium chloride, azastene, azastrptonigrin, azatodine, azathioprine, azauridine, azelastine, azepexole, azepindole; azetepa, azidamfenicol, azidocillin, azimexon, azintamide, azipramine, azithromycin, azlocillin, azolimine, azosemide, azotomycin, aztreonam, azumolene, bacampicillin, baclofen, bacmecillinam, balsalazide, bamaluzole, bambuterol, bamethan, bamifylline, bamipine, bamnidazole, baquiloprim, barbexaclone, barbital, barucainide, batilol, bazinaprine, becanthone, beclamide, beclobrate, beclomethasone dipropionate, beclotiamine, befiperide, befunolol, befuraline, bekanamycin, belarizine, beloxamide, bemarinone, bemegride, bemetizide, bemitradine, benactyzine, benafentrine, benanserin, benapryzine, benaxibine, benazepril, bencianol, bencisteine, benclonidine, bencyclane, bendamustine, bendazac, bendazol, benderizine, bendroflumethiazide, benethamide penicillin, benexate, benflorex, benfosformin, benfotiamine, benfurodil hemisuccinate, benhepazone, benidipine, benmoxin, benolizime, benorilate, benorterone, benoxafos, benoxaprofen, benoxinate, benperidol, benproperine, benrixate, bensalan, benserazide, bensuldazic acid, bentazepam, bentemazole, bentiamine, bentipimine, bentiromide, benurestat, benzaldehyde, benzalkonium chloride, benzaprinoxide, benzarone, benzbromarone, benzestrol, benzethidine, benzethonium chloride, benzetimide, benzilonium bromide, benzindopyrine, benziodarone, benzmalecene, benznidazole, benzobarbital, benzocaine, benzoclidine, benzoctamide, benzodepa, benzododecinium chloride, benzoic acid, benzoin, benzonatate, benzopyrronium bromide, benzoquinium chloride, benzotript, benzoxiquine, benzoxonium chloride, benzoyl peroxide, benzoylpas, benzphetamine, benzpiperylon, benzpyrinium bromide, benzquercin, benzquinamide, benzthiazide, benztropine, benzydamine, benzylpenicillin, benzylsulfamide, beperidium iodide, bephenium naphtoate, bepiastine, bepridil, beraprost, berberine sulfate, bermastine, bermoprofen,

berythromycin, besulpamide, beslunide, beta carotene, betacetylmethadol, betahistine, betaine, betameprodine, betamethadol, betamethasone, betamethasone acetate, betamethasone acibutate, betamethasone benzoate, betamethasone dipropionate, betamethasone phosphate, betamethasone valerate, betamicin, betaprodine, betaxolol, betazole, bethanechol chloride, bethanidine, betiatide, betoxycaine, bevantolol, bevonium metilsulfate, bezafibrate, bezitramide, bialamicol, bibenzonium bromide, bibrocathol, bicifadine, biclodil, biclofibrate, biclotymol, bicozamycin, bidimazium iodine, bietamiverine, bietaserpine, bifemelane, bifepramide, bifluranol, bifonazole, binedaline, binfloxacin, binfibrate, bioallethrin, bioresmethrin, biotin, bipenamol, biperiden, biphenamine, biriperone, bisacodyl, bisantrene, bis(aziridinyl) butanediol, bisbendazole, bisbentiamine, bisfenazone, bisfentidine, bismuth betanaphthol, bismuth-triglycollamate, bismuth subgallate, bismuth subsalicylate, bisorbin, bisoprolol, bisorcic, bioxatin acetate, bispyrithione magsulfex, bithionol, bithionoloxide, bitipazone, bitoterol, bitoscantate, bleomycin, bluensomycin, bofumustine, bolandiol dipropionate, bolasterone, bolazine, boldenone undecylenate, bolenol, bolmantalate, bometolol, bopindolol, bornaprine, bornaprolol, bornelone, botiacrine, boxidine, brallobarbital, brazergoline, brefonalol, bremazocine, brequinar, bretylium tosylate, brindoxime, brivundine, brobactam, broclepride, brocresine, brocrinat, brodimoprim, brofaromine, brofezil, brofoxine, brolaconazole, brolamfetamine, bromacrylide, bromadoline, bromamid, bromazepam, bromchlorenone, bromebric acid, bromerguride, brometenamine, bromfenac, bromhexine, bromindione, bromisovalum, bromociclen, bromocriptine, bromodiphenhydramine, bromofenofos, bromopride, bromoxandide, bromperidol, bromperidol decanoate, brompheniramine, bronopol, broparestrol, broperamole, bropirimine, broquinaldol, brosotamide, brosuximide, brotianide, brotizolam, brovanexine, brovincamine, broxaldine, broxaterol, broxitalamic acid, broxuridine, broxyquinoline, bruceantin, brucine, bucainide, bucetin, buciclovir, bucillamine, bucindolol, bucladesine, buclizine, buclosamide, bucloxic acid, bucolome, bucricaine, bucromarone, bucrylate, bucumolol, budesonide, budipine, budotitane, budralazine, bufenadrine, bufeniode, bufetolol, bufexamac, bufezolac, buflomedil, bufogenin, buformin, bufrolin, bufuralol, bumadizone, bumecaine, bumepidil, bumetanide, bumetrizole, bunaftine, bunamidine, bunamiodyl, bunaprolast, bunazosin, bunitrolol, bunolol, buparvaquone, bupicomide, bupivacaine, bupranolol, buprenorphine, bupropion, buquineran, buquinolate, buquiterine, buramate, burodiline, buspirone, busulfan, butabarbital, butacaine, butacetin, butaclamol, butadiazamide, butafosfan, butalamine, butalbital, butamben, butamirate, butamisole, butamoxane, butanediol cyclic sulfite, butanilicaine, butanixin, butanserin, butantrone, butaperazine, butaprost, butaverine, butedronate, buterizine, butetamate, butethamine, buthiazide, butibufen, butidrine, butikacin, butilfenin, butinazocine, butinoline, butirosin, butixirate, butobendine, butoconazole, butocrolol, butoctamide, butofilolol, butonate, butopamine, butopiprine, butoprozine, butopyrammonium iodide, butorphanol, butoxamine, butoxylate, butriptyline, butropium bromide, butylated hydroxyanisole, butylated hydroxytoluene, butylparaben, butynamine, buzepide metiodide, cabastine, cabergoline, cadralazine, cafaminol, cafedrine, caffeine, calcifediol, calcitrol, calcium citrate, calcium dobesilate, calcium glubionate, calcium gluceptate, calcium gluconate, calcium glycerophosphate, calcium hypophosphite, calcium lactate, calcium lactobionate, calcium levulinate, calcium mandelate, calcium pantothenate, calcium phosphate dibasic, calcium phophate tribasic, calcium saccharate, calcium stearate, calusterone, camazepam, cambendazole, camiverine, camostast, camphotamide, camptothecin, camylofin, canbisol, cannabinol, canrenoic acid, canrenone, cantharidine, capobenic acid, capreomycin, caproxamine, capsaicine, captamine, captodiame, captopril, capuride, caracemide, caramiphen, carazolol, carbachol, carbadox, carbaldrate, carbamazepine, carbamide peroxide, carbantel lauryl sulfate, carbaril, carbarsone, carbaspirin calcium, carbazeran, carbazochrome, carbazachrome salicylate, carbazachrome sulfonate, carbazocine, carbeniciltin,

carbenicillin indanyl, carbencillin phenyl, carbenoxolone, carbenzide,
carbestrol, carbetapentane, carbidopa, carbimazole, carbinoxamine, carbiphene,
carbocloral, carbocysteine, carbofenotion, carbol-fuschin, carbomycin,
carboplatin, carboprost, carboprost methyl, carboquone, carbromal, carbubarb,
carburazepam, carbutamide, carbuterol, carcainium chloride, carebastine,
carfentanil, carfimate, carisoprodol, carmantadine, carmetizide, carmofur,
carmustine, carnidazole, carnitine, carocainide, caroverine, caroxazone,
carperidine, caperone, carphenazine, carpindolol, carpiramine, carprofen,
carpronium chloride, carsalam, cartazolate, carteolol, carubicin, carumonam,
carvedilol, carzenide, carzolamide, cathine, cathinone, cefaclor, cefadroxil,
cefalonium, cefaloram, cefamandole, cefamandole naftate, cefaparole,
cefatrizine, cefazaflur, cefazedone, cefazolin, cefbuperazone, cefcanel,
cefcanel daloxate, cefedrolor, cefempidone, cefepime, cefetamet, cefetrizole,
cefvitril, cefixime, cefmenoxime, cefmepidium chloride, cefmetazole, cefminox,
cefodizime, cefonizid, cefoperazone, ceforanide, cefotaxime, cefotetan,
cefotiam, cefoxazole, cefoxitin, cefpimizole, cefpiramide, cefpirome,
cefpodoxime, cefpodoxime proxetil, cefquinome, cefrotil, cefroxadine,
cefsulodin, cefsumide, ceftazidime, cefteram, ceftezole, ceftiofur, ceftiolene,
ceftioxide, ceftizoxime, ceftriaxone, cefuracetime, cefuroxime, cefuraxime
axetil, cefurzonam, celiprolol, cephacetrile, cephalexin, cephaloglycin,
cephaloridine, cephalothin, cephapirin, cephradine, cetaben, cetamolol,
cethexonium chloride, cetiedil, cetirizine, cetocycline, cetohexazine,
cetophenicol, cetotiamine, cetoxime, cetraxate, chaulmosulfone, chendiol,
chiniofon, chlophedianol, chloracyzine, chloral betaine, chloral hydrate,
chloralose, chlorambucil, chloramine, chloramphenicol, chloramphenicol
palmitate, chloramphenicol succinate, chlorazanil, chlorbenzoxamine,
chlorbetamide, chlorcyclizine, chlordantoin, chlordiazepoxide, chlordimorine,
chlorhexadol, chlorhexidine, chlorhexidine phosphanilate, chlorindanol,
chlorisondamine chloride, chlormadinone acetate, chlormerodrin, chlormezanone,
chlormidazole, chloronaphazine, chloroazodin, chlorobutanol, chlorocresol,
chlorodihydroxyandrostenone, chloroethyl mesylate,
5-chloro-3'-fluoro-2'3-dideoxyuridine, chloroguanide, chlorophenothane,
chloroprednisone acetate, chloroprocaine, chloropyramine, chloroquine,
chloroserpidine, chlorothen, chlorothiazide, chlorotriansene, chloroxine,
chloroxylenol, chlorozotocin, chlorphenesin, chlorphenesin carbamate,
chlorpheniramine, chlorphenoctium amsonate, chlorphenoxamine, chlorphentermine,
chlorproethazine, chlorproguanil, chlorpromazine, chlorpropamide,
chlorprothixene, chlorquinaldol, chlortetracycline, chlorthalidone,
chlorthenoxazine, chlorzoaxazone, chloecalciferol, cholic acid, choline
chloride, choline glycerophosphate, chromocarb, chromonar, ciadox, ciamexon,
cianergoline, cianidol, cianopramine, ciapilome, cicaprost, cicarperone,
ciclactate, ciclafrine, ciclazindol, cicletanine, ciclomenol, ciclonicate,
ciclonium bromide, ciclopirox, ciclopramine, cicloprofen, cicloprolol,
ciclosidomine, ciclotizolam, ciclotropium bromide, cicloxilic acid,
cicloxolone, cicortonide, cicrotic acid, cidoxepin, cifenline, cifostodine,
ciglitazone, ciheptolane, ciladopa, cilastatine, cilazapril, cilazaprilat,
cilobamine, cilofungin, cilostamide, cilostazol, ciltoprazine, cimaterol,
cimemoxin, cimepanol, cimetidine, cimetropium bromide, cimoxatone, cinchonine,
cinchophen, cinecromen, cinepaxadil, cinepazet, cinepazic acid, cinepazide,
cinfenine, cinfenoac, cinflumide, cingestol, cinitapride, cinmetacin,
cinnamaverine, cinnamedrine, cinnarizine, cinnarizine clofibrate,
cinnofuradione, cincotramide, cinodine, cinolazepam, cinoquidox, cinoaxin,
cinoxate, cinoxolone, cinooxopazide, cinperene, cinprazole, cinpropazide,
cinromide, cintazone, cintriamide, cinperone, ciprafamide, ciprafazone,
ciprefadol, ciprocinonide, ciprofibrate, ciprofloxacin, cipropride,
ciproquazone, ciprostene, ciramadol, cirazoline, cisapride, cisconazole,
cismadinone, cisplatin, cistinexine, citalopram, citatepine, citenamide,
citenazone, citicoline, citiolone, clamidoxic acid, clamoxyquin, clanfenur,
clanobutin, clantifen, clarithromycin, clavulanic acid, clazolam, clazolimine,

clazuril, clebopride, clefamide, clemastine, clemeprol, clemizole, clenbuterol, clenpirin, cletoquine, clibucaine, clidafidine, clidanac, clidinum bromide, climazolam, climbazole, climiqualine, clindamycin, clindamycin palmitate, clindamycin phosphate, clinofibrate, clinolamide, cliquinol, clioxanide, clipoxamine, cliprofen, clobazam, clobenoside, clobenzepam, clobenzorex, clobenztropine, clobetasol propionate, clobetasone butyrate, clobutinol, clobuzarit, clocanfamide, clocapramine, clociguanil, clocinizine, clocortolone acetate, clocortolone pivalate, clocoumarol, clodacaine, clodanolene, clodazon, clodoxopone, clodronic acid, clofazimine, clofenamic acid, clofenamide, clofenciclan, clofenetamine, clofenoxyde, clofenvinfos, clofeverine, clofexamide, clofezone, clofibrate, clofibric acid, clofibride, clofilium phosphate, cloflucarban, clofoctol, cloforex, clofurac, clogestone acetate, cloguanamil, clomacran, clomegestone acetate, clometacin, clometherone, clomethiazole, clometocillin, clomifenoxide, clominorex, clomiphene, clomipramine, clomocycline, clomoxir, clonazepam, clonazoline, clonidine, clonitazene, clonitrate, clonixeril, clonixin, clopamide, clopenthixol, cloperastine, cloperidone, clopidogrel, clopidol, clopimozide, clopipazan, clopirac, cloponone, cloprednol, cloprostenol, cloprothiazole, cloquinate,

US-PAT-NO: 6251628

DOCUMENT-IDENTIFIER: US 6251628 B1

TITLE: Isolated nucleic acid molecules encoding Smad7

DATE-ISSUED: June 26, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Nakao; Atsuhito	Chiba	N/A	N/A	JP
Heldin; Carl-Henrik	Uppsala	N/A	N/A	SE
Dijke; Peter Ten	Uppsala	N/A	N/A	SE

APPL-NO: 09/ 082092

DATE FILED: May 20, 1998

PARENT-CASE:

RELATED APPLICATIONS

This application claims priority under 35 U.S.C. .sctn.119 from U.S. provisional application serial No. 60/047,221, filed May 20, 1997, from U.S. provisional application serial No. 60/060,465, filed Sep. 30, 1997, from U.S. provisional application serial No. 60/075,940, filed Feb. 25, 1998, and from U.S. provisional application serial No. 60/077,033, filed Mar. 6, 1998.

US-CL-CURRENT: 435/69.1, 435/243 , 435/320.1 , 435/325 , 435/410 , 536/23.5

ABSTRACT:

The invention describes nucleic acids encoding the Smad7 protein, including fragments and biologically functional variants thereof. Also included are polypeptides and fragments thereof encoded by such nucleic acids, and antibodies relating thereto. Methods and products for using such nucleic acids and polypeptides also are provided.

18 Claims, 70 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 42

---------- KWIC ---------

Detailed Description Text - DETX (74):

The assay mixture also comprises a candidate pharmacological agent. Typically, a plurality of assay mixtures are run in parallel with different agent concentrations to obtain a different response to the various concentrations. Typically, one of these concentrations serves as a negative control, i.e., at zero concentration of agent or at a concentration of agent below the limits of assay detection. Candidate agents encompass numerous chemical classes, although typically they are organic compounds. Preferably, the candidate pharmacological agents are small organic compounds, i.e., those having a molecular weight of more than 50 yet less than about 2500, preferably

less than about 1000 and, more preferably, less than about 500. Candidate agents comprise functional chemical groups necessary for structural interactions with polypeptides and/or nucleic acids, and typically include at least an amine, carbonyl, hydroxyl or carboxyl group, preferably at least two of the functional chemical groups and more preferably at least three of the finctional chemical groups. The candidate agents can comprise cyclic carbon or heterocyclic structure and/or aromatic or polyaromatic structures substituted with one or more of the above-identified finctional groups. Candidate agents also can be biomolecules such as peptides, saccharides, fatty acids, sterols, isoprenoids, purines, pyrimidines, derivatives or structural analogs of the above, or combinations thereof and the like. Where the agent is a nucleic acid, the agent typically is a DNA or RNA molecule, although modified nucleic acids as defined herein are also contemplated.

Detailed Description Text - DETX (131):

To investigate whether Smad7 modulates the responsiveness to TGF-.beta., the TGF-.beta.-inducible luciferase p3TPLux reporter construct, which contains the TGF-.beta.-inducible PAI-1 promoter, was transfected into MV1Lu mink epithelial cells in the absence or presence of Smad7 cDNA. Smad7 was found to exert a dose-dependent inhibition TGF-.beta.-induce luciferase activity (FIG. 2a). Moreover, the induction of p3TPLux luciferase by a constitutively active variant of T.beta.R-I, when transfected in R-mutant cells, was also inhibited by cotransfection with Smad7, as was the response by a constitutively active variant of the structurally related type I receptor for activin (ActR-IB) (FIG. 2B). Transfection of Smad2 did not affect TGF-.beta.1-induced p3TPLux luciferase response in Mv1Lu cells (FIG. 2a). This inhibitory effect was specific as Smad7 did not inhibit the phorbol 12-myristate 13-acetate (PMA)/epidermal growth factor-induced p3TPLux luciferase response. In addition, the forskolin-mediated transcriptional induction using a cAMP-responsive-element-containing reporter construct was not affected by Smad7. These results indicate that Smad7 is a potent negative regulator of both T.beta.R-I- and ActR-IB-induced p3TPLux response.

US-PAT-NO: 6239264

DOCUMENT-IDENTIFIER: US 6239264 B1

TITLE: Genomic DNA sequences of ashbya gossypii and uses thereof

DATE-ISSUED: May 29, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Philippsen; Peter	Riehen	N/A	N/A	CH
Pohlmann; Rainer	Lorrach	N/A	N/A	DE
Steiner-Lange; Sabine	Bonn	N/A	N/A	DE
Mohr; Christine	Allschwil	N/A	N/A	CH
Wendland; Jurgen	Lorrach	N/A	N/A	DE
Knechtle; Philipp	Oberwil	N/A	N/A	CH
Rebischung; Corinne	Saint-Louis	N/A	N/A	FR

APPL-NO: 08/ 998416

DATE FILED: December 24, 1997

US-CL-CURRENT: 536/23.1, 435/320.1 , 536/24.3 , 536/24.32

ABSTRACT:

The present invention relates to the terminal sequencing of random genomic fragments performed with the filamentous fungus A.gossypii, to the sequences obtained therewith and the use of the sequences for forensic identification, to characterize genes and gene organization of this ascomycete by inter-genomic comparison, to identify biosynthetic genes that can be used as selection markers, to isolate promotors and terminators for application in a homologous as well as heterologous context, to find putative centromere containing clones, chromosome mapping, chromosome identifying, general information about chromosome organization and in addition to identify ORF containing SRS sequences with no homology to S. cerevisiae or any other organism which allows the identification of A. gossypii specific genes.

2 Claims, 0 Drawing figures

Exemplary Claim Number: 1

---------- KWIC ---------

Detailed Description Paragraph Table - DETL (16):
PAG1634UP 4 PAG1635RP YDL052c SLC1 Fatty acyltransferase 1 two genes covered by RP- SRS,same as PAG1664 YLR377c FBP1 Fructose-1,6-bisphophatase 1 two genes covered by RP- SRS,same as PAG1664 PAG1635UP YDL054c putative transmembrane protein 1 same as PAG1664 PAG1636RP YLL055w protein with sim to DAL5 and members of 1 the allantoate perinease family of the major facilitator superfamily (MFS) PAG1636UP YKL215c protein with sim to Pseudomonas 1 hydantoinases hyuA-hyuB PAG1637RP YER157w unknown function 2 syntenie,same as PAG1060 PAG1637UP YER155c BEM2 bud-emergence protein 1 codons 403 to 218 including N- term+promoter(?)syntenie YER156c:unknown function,same as PAG1060 PAG1638RP YCL037c with sim to SLF1, has a motif in

common 1 syntenie.YCL038C:unknown with conserved sequence in LHP1 but does function, len 528aa37c:is SRO9 not contain a RNA recognition motif PAG1638UP YCL039w probably a member of the WD-40 family 1 syntenie PAG1639RP YKL046c unknown function, has 2 predicted TMDs 1 PAG1639UP YMR020w FMS1 sim to corticosteroid-binding protein 1 PAG1640RP 4 PAG1640UP YLR196w PWP1 member of WD-40 repeat family 1 PAG1642RP YKR023w unknown function 1 syntenie PAG1642UP YKR024c unknown function, probable purine 1 syntenie nucleotide-binding protein PAG1643RP YMR179w SPT21 protein that amplifies the magnitude of 1 syntenie; YMR180c:len 320, transcriptional regulation unknown at various loci PAG1643UP YMR181c unknown function 1 syntenie PAG1644RP 4 PAG1645RP YDR089w unknown function ; with leucine zipper 1 syntenie, two genes covered by pattern RP-SRS ending syntenie YER161c SPT2 HMG-like chromatin protein that interacts 1 syntenie, two genes covered by with SNF1p through a conserved domain RP-SRS ending syntenie PAG1645UP YDR088c SLU7 pre-mRNA splicing factor affecting 3' 1 syntenie splice site choice, required only for the second catalytic step PAG1646RP YML120c NDI1 NADH-ubiquinone oxidoreductase 1 PAG1646UP YHR190w ERG9 Squalene synthtase(farnesyl-diphosphate 1 farnesyltransferase), branch point for isoprenoid biosynthesis pathway PAG1647RP YML004c GLO1 sim to glyoxalases 1 PAG1647UP YNR011c PRP2 RNA-dependent ATPase of DEAD box 1 family required for first catalytic event of pre-mRNA splicing PAG1648RP 4 PAG1648UP YNR016c FAS3 first and rate limiting step in fatty acid 1 biosynthesis pathway PAG1649RP YHR206W SKN7 Transcription factor with homology 1 toresponse regulator proteins of bacterial two-component systems and DNA-binding region of Hsf1p, may be involved in the response to oxidative stress. May act in parallel to PKC1-MAP kinase pathway to regulate growth at the cell surface, but is not in the sane pathway as PKC1, null mutant w/O phenotype, high level of overexpression is lethal: Has a potential coiled-coil domain PAG1649UP YER183c unknown function 1 syntenie; two genes covered by UP-SRS YER182c unknown, but essential 1 syntenie, two genes covered by UP-SRS PAG1650RP YOR317w FAA1 :long-chain fatty acid CoA ligase (fatty acid 1 activator 1), can incorporate exogenous myristate into myristoyl-CoA and other fatty acids to the CoA derivatives PAG1650UP YMR100W unknown function 1 PAG1651RP YNL121c TOM70 MT specilized import receptor of the outer 1 syntenie membrane, has tetratricopeptide repeats PAG1651UP YNL123w unknown function 1 syntenie, two genes covered by UP-SRS YNL122c unknown function 1 syntenie; two genes covered by UP-SRS PAG1652RP YOL095c sim to DNA helicase pcrA 1 syntenie PAG1652UP YOL094c RFC4 Replication Factor C , 37kD subunit 1 syntenie PAG1653RP YHR047c AAP1 see ATP8,ORC6;Highly similar to 1 aminopeptidase yscII (S. cerevisiae), AMPE_MOUSE, and several other zinc metalloproteases PAG1653UP YHR074w Weak similarity to spore outgrowth factor B 1 (sporulation protein OUTB, B. subtilis) PAG1654RP YMR196w unknown function 1 very end of the gene PAG1654UP YMR196w unknown function 1 very start of gene, w/o promoter PAG1655RP YNL202w SPS19 sporulation specific protein, probably 1 syntenie peroxisomal, ends in SKL* PAG1655UP YNL200c contains a possible signal-peptide, predicted 1 syntenie; two genes covered by to be extracellular UP-SRS YNL201c protein involved in the regulation of carbon 1 syntenie; two genes covered by metabolism UP-SRS PAG1656RP YHR201c PPX1 degrades polyphosphate, converting ADP to 1 ATP PAG1656UP YJR141w unknown protein 1 PAG1657RP YJL130c URA2 multifunctional pyrimidine biosynthesis 1 from codon 1781 to the C- protein terminus PAG1657UP YKR051w unknown protein 1 PAG1659RP YBR038w CHS2 Chitin Synthase II, responsible for primary 1 syntenie septum disk; Mutants resistant to calcofluor white, 8 TMD, PAG1659UP YBR037c SCO1 mutant is unable to grow on non- 1 syntenie fermentable c-sources Membrane location is altered in an rho0 strain PAG1660RP YBL004w unknown function; has 12 TMD 1 codons 800-1033 PAG1660UP YBL004w 1 codons 2167-1951; C-terminus missing PAG1664RP YDL052c SLC1 1 syntenie, same as PAG1635 PAG1664UP YDL054c unknown function, putative transmembrane 1 syntenie; same as PAG1635 protein PAG1666RP MITO- DNA

PAG1666UP MITO- DNA PAG1667RP YMR061w RNA14 component of pre-mRNA 3' end processing 1 same as PAG1611 factor involved in poly(A) site choice, interacts with Rna15p, Fip1p, and Pap1p PAG1667UP YKL075c unknown protein 1 same as PAG1611 PAG1669RP YLR277C BRR5 protein required for processing of mRNA 3' 1 syntenie end PAG1669UP YLR281c unknown, however there are other 1 syntenie; YLR278c:protein with overlapping ORF's sim to transcription factors, has Zn(2)- Cys(6) fungal- type binuclear cluster domain in the N-terminal region, len 1341aa PAG1670RP YJL191w CRY2 ribosomal protein rp59 (E. coli S11, rat and 1 syntenie; two genes covered by human S14) RP-SRS, same as PAG1603 YJL190c RPS24A RPS24A:ribosomal protein RPS24 (E. coli 1 syntenie, same as PAG1603 S8, mammalian S24) PAG1670UP YJL069c unknown protein 1 same as PAG1603 PAG1671RP YBR112c SSN6 has 10 TPR repeats (TPR- 2 Tetratricopeptide) PAG1671UP YML042w CAT2 Carnitine-o-acetyltransferase, peroxisomal 1 codons 442 to 655 promoter and and mitichondrial, not required for growth terminator missing; regulatable on fatty acids,Catalytic activity: undetected promoter? in cells grown on glucose, increased on glycerol or acetate, very high on oleate PAG1672RP 4 sp.vertline.P24197.vertline.YGID_ECOLI HYPOTHETICAL 28.3 KD PROTEIN IN T . . . -3 73 1.2e- 10 5 PAG1672UP YDL203c unknown, has weak sim to SKT5 1 PAG1673RP YDL104c QRI7 sim to E. coli orfX gene; may be in a cold 1 syntenie spot for recombination YDL105w QRI2 unknown 1 syntenie; two genes covered by RP-SRS PAG1673UP YMR166c sim to members of the MCF MT carrier 1 not in syntenie to RP-SRS protein family PAG1674RP YPL072w LPF12 unknown function 1 syntenie PAG1674UP YPL075w GCR1 required for expression of glycolytic genes. 1 syntenie; binds to DNA with high affinity but low YPL074w:YTA6:CDC48- specificity , motif CTTCC, contains a ATPase- family leucine zipper that is necessary and sufficient for homodimerization PAG1675RP YMR259C unknown, has sim to YGR273p 1 PAG1675UP YML100w TSL1 alternate third subunit of the trehalose-6- 1 phosphate synthase complex, probably regulatory PAG1676RP YLR429W unknown, with WD-40 repeats 1

US-PAT-NO: 6057298

DOCUMENT-IDENTIFIER: US 6057298 A

TITLE: Keratin K1 expression vectors and methods of use

DATE-ISSUED: May 2, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Roop; Dennis R.	Houston	TX	N/A	N/A
Rothnagel; Joseph A.	Houston	TX	N/A	N/A
Greenhalgh; David A.	Houston	TX	N/A	N/A
Yuspa; Stuart H.	Bethesda	MD	N/A	N/A

APPL-NO: 08/ 452872

DATE FILED: May 30, 1995

PARENT-CASE:

RELATED APPLICATION

This application is a divisional of application Ser. No. 08/147,777, Roop et al., filed Nov. 1, 1993, U.S. Pat. No. 5,914,265, entitled "Keratin K1

Expression Vectors and Methods of Use; which is a continuation-in-part of Roop et al., Ser. No. 08/145,387 filed Oct. 29, 1993, now abandoned, entitled "Keratin K1 Expression Vectors and Methods of Use," which is also a continuation-in-part of Roop et al., U.S. patent application Ser. No. 07/876,289, filed Apr. 30, 1992, now abandoned, entitled "Development of a Vector to Target Gene Expression to the Epidermis of Transgenic Animals," the whole of which (including drawings) are all hereby incorporated by reference. This divisional application is also related to Roop et al., U.S. Ser. No. 07/876,286, filed Apr. 30, 1992, now abandoned, entitled "Constitutive and Inducible Epidermal Vector Systems," and its continuation-in-part application by Roop et al., Ser. No. 08/145,388, entitled "Specific Expression Vectors and Methods of Use," filed Oct. 29, 1993, now abandoned, and its continuation-in-part application by Roop et al., Ser. No. 08/146,930, entitled "Specific Expression Vectors and Methods of Use," filed Nov. 1, 1993, U.S. Pat. No. 5,958,764, all (including drawings) hereby incorporated by reference herein.

US-CL-CURRENT: 514/44, 435/320.1 , 435/371 , 435/375

ABSTRACT:

A keratin K1 vector for expression of a nucleic acid sequence in an epidermal cell. The vector includes a 5' flanking region which includes necessary sequences for expression of a nucleic acid cassette, a keratin K1 3' flanking region which regulates expression of a nucleic acid sequence, predominantly in the epidermis, and a linker which connects the 5' flanking region to a nucleic acid. The linker has a position for inserting a nucleic acid cassette. The linker does not contain the coding sequence of a gene that the linker is naturally associated with. That is, the linker is not the normal gene associated with the 5' and 3' regions.

23 Claims, 10 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 7

---------- KWIC ---------

Detailed Description Text - DETX (172):
To assess whether the sequences protected in FP(A) and FP(B) were functionally involved in mediating the calcium response, a reporter construct was employed consisting of the CaRE linked to a SV40 minimal promoter CAT construct. The CaRE.CAT construct has been shown to be activated by increasing concentrations of calcium in the medium (Rothnagel, et al., J. Invest. Dermatol., Vol. 101, pp. 506-513 (1993)). To test whether endogenous AP-1 factors were able to induce CAT activity from the CaRE.CAT construct we added 12-0-tetradecanoylphorbol-13-acetate (TPA) to transfected keratinocytes. TPA is a potent inducer of AP-1 factors in keratinocytes (Dotto, et al., EMBO J., Vol. 5, pp. 2853-2857 (1986)), and is able to activate CaRE.CAT in a dose dependent manner. Thus both calcium and TPA are able to induce CAT activity from the CaRE.CAT construct.

Other Reference Publication - OREF (110):
Vahlquist et al., "Vitamin A and .beta.-Carotene Concentrations at Different Depths of the Epidermis: A Preliminary Study in the Cow Snout," 92 Upsala J. Med. Sci. 253, 1987.

US-PAT-NO: 5972357

DOCUMENT-IDENTIFIER: US 5972357 A

TITLE: Healthy foods and cosmetics

DATE-ISSUED: October 26, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Yamaguchi; Fumio	Noda	N/A	N/A	JP
Saito; Makoto	Noda	N/A	N/A	JP
Ishikawa; Hiroharu	Noda	N/A	N/A	JP
Kataoka; Shigehiro	Noda	N/A	N/A	JP
Ariga; Toshiaki	Noda	N/A	N/A	JP

APPL-NO: 08/ 975713

DATE FILED: November 21, 1997

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
JP	8-353869	December 19, 1996
JP	9-199119	July 10, 1997
JP	9-199120	July 10, 1997

US-CL-CURRENT: 424/401, 514/675 , 514/678 , 514/690 , 514/724 , 514/729 , 514/730

ABSTRACT:

The present invention relates to healthy foods and cosmetics. More particularly, it relates to healthy foods and cosmetics containing a polyisoprenylated benzophenone derivatives as effective ingredients and having a variety of functions for maintaining health such as anti-ulcer activity, the Maillard reaction inhibiting activity, anti-oxidation activity, reactive oxygen species scavenging activity, and anti-tumor promotion activity.

5 Claims, 0 Drawing figures

Exemplary Claim Number: 1

---------- KWIC ---------

Detailed Description Text - DETX (13):

The anti-oxidation activities was evaluated according to the method by A. Ben Aziz et al. (see Phytochemistry, 10, 1445, 1971). That is to say, an emulsion of linoleic acid and .beta.-carotene was first prepared with Tween 20 (Sigma) and adjusted to pH 7.0 with 0.1 M trishydroxyaminomethane-hydrochloride. To the emulsion was added 0.0005% of a substance to be tested, and the mixture was reacted at 25.degree. C.

Detailed Description Text - DETX (14):

During the reaction, the color degradation of .beta.-carotene in association with the autoxidation of linoleic acid was measured with the passage of time as

the decreased value of the optical density at 460 nm with a spectrophotometer.

Detailed Description Text - DETX (19):

The oxidation process of linoleic acid in Experimental Example 1 is promoted by a metal ion. If 5 .mu.M of copper sulfate is added to the reaction system, the color-degradation rate of .beta.-carotene is promoted by about 30%. When garcinol was added to this system, the ratio of the decreasing rate of absorbance was 0.57. In this connection, it was 0.13 for vitamin E and 0.84 for (+)-catechin. Thus, garcinol is revealed to have a strong antioxidant activity in the lipid oxidation process catalyzed by the metal ion.

Detailed Description Text - DETX (53):

The EBV potentially infected human lymphoblasts (Raji) were first prepared in a concentration of 5.times.10.sup.5 cell/ml, and cultured in an RPMI-1640 medium to which 3 mM n-butyric acid (inducer) and 50 nM TPA (12-O-tetradecanoylphorbol-13-acetate) (promoter) were added in an atmosphere of 5% carbon dioxide and 95% air under at a temperature of 37.degree. C. for 48 hours. The cells in which EBV-EA had been induced were detected microscopically by indirect immunofluorescence with the serum of a nasopharyngeal carcinoma (NPC) patient. In this system, various concentrations (8 .mu.g/ml, 40 .mu.g/ml, and 200 .mu.g/ml) of the test substance (garcinol sample shown in Referential Example 1) dissolved in DMSO (dimethylsolfoxide) were added together with the promoter to the cells. The activity of suppressing the induction of the Epstein-Barr virus early antigen was defined as the decreasing rate of the cells in which EBV-EA had been induced.

Detailed Description Paragraph Table - DETL (6):

Garcinia indica extract	50.0
.beta.-carotene	20.0
.alpha.-dl-tocopherol	0.75
DHA	30.0
olive oil	899.25

US-PAT-NO: 5837728

DOCUMENT-IDENTIFIER: US 5837728 A

TITLE: 9-cis retinoic acid esters and amides and uses thereof

DATE-ISSUED: November 17, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Purcell; William P.	Memphis	TN	N/A	N/A

APPL-NO: 08/ 380011

DATE FILED: January 27, 1995

US-CL-CURRENT: 514/529, 514/559 , 554/221

ABSTRACT:

Esters and amides of 9-cis-retinoic acid are synthesized, formulated into pharmaceutically acceptable carriers and administered for the treatment of acne vulgaris, cystic acne, hyper-pigmentation, hypo-pigmentation, psoriasis, dermal and epidermal hypoplasia and kerotoses, the reduction of wrinkling of the skin as an incident of aging and actinic damage, normalization of the production of sebum, the reduction of enlarged pores, promoting the rate of wound healing, limiting of scar tissue formation during healing and the like. They are additionally useful for treatment or amelioration of the same additional classes of skin disorders as is retinoic acid itself and other retinoids. These disorders include ichthyoses (e.g., ichthyosis hystrix, epidermolytic hyperkeratosis, and lamellar ichthyosis), follicular disorders (e.g., pseudofolliculites, senile comedones, nevus comidonicas, and trichostatis spinulosa), benign epithelial tumors (e.g., flat warts, trichoepithelioma, and molluscum contagiosum), perforated dematoses (e.g., elastosis perforans seripiginosa and Kyrles disease), and disorders of keratinization (e.g., Dariers disease, keratoderma, hyperkeratosis plantaris, pityriasis rubra pilaris, lichen planus acanthosis nigricans, and psoriasis). The esters and amides of 9-cis-retinoic acid are also effective for the non-irritating treatment of effects attributable to aging and particularly to photodamage and photoaging. The use of these compounds extends to non-irritating treatments involving the retardation and reversal of additional dermal and cosmetic conditions which are ameliorated by tretinoin such as the effacement of wrinkles, improvement in appearance, namely color and condition of the skin, spots caused from exposure to the sun as well as other skin disorders. The esters and amides of 9-cis-retinoic acid are exceptionally active when compared to other retinoids employed for such indications, and are also exceptionally safe in effective therapeutic doses in contrast to other retinoids.

40 Claims, 0 Drawing figures

Exemplary Claim Number: 1,12

---------- KWIC ---------

Brief Summary Text - BSTX (10):
.beta.-Carotene, as a Vitamin A precursor has also been explored, with the

expectation of greater safety. The precursor is less effective, however, since it is itself largely inactive and must be cleaved to the active Vitamin form before the desired effects are produced, and the cleavage is difficult to manage, predict and control.

Detailed Description Text - DETX (63):

The ODC/Retinoid Bioassay is based on the method of Verma, A. K. and Boutwell, R. K., Cancer Res. (1977) 37:2196-2201. The ODC assay measures a compounds effect on the prevention of the induction of ODC, namely the effect of the retinoid compound on the inhibition of the tumor promoter 12-O-tetradecanoylphorbol-13-acetate (TPA) induced ODC activity. The assay is carried out using CD-1 mice (aged 7 to 9 weeks). The dorsal hair of the mice is shaved 3-4 days before testing. Four mice are used for each point. The test retinoids, at one of two dose levels (1.7 and 17 nmoles) dissolved in 0.2 ml of acetone is applied topically to the back of each shaved mouse. A single dose of TPA (17 nM) is applied to the back of each treated mouse 30 minutes later. Control groups are treated with either acetone alone, TPA, or tretinoin. The mice are sacrificed by cervical dislocation 5 hours after TPA treatment.

US-PAT-NO: 5475029

DOCUMENT-IDENTIFIER: US 5475029 A

TITLE: Farnesyl compounds as cholesterol lowering agents

DATE-ISSUED: December 12, 1995

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Bradfute; David L.	Wooster	OH	N/A	N/A
Simoni; Robert D.	Palo Alto	CA	N/A	N/A

APPL-NO: 08/ 088698

DATE FILED: July 8, 1993

US-CL-CURRENT: 514/549, 514/722 , 514/739 , 560/205 , 568/687

ABSTRACT:

Farnesyl derivatives, particularly farnesyl acetate, are used to lower the 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase activity in cells, thereby reducing cholesterol biosynthesis. The compounds may be administered to hypercholesterolemia patients to reduce the overall level of serum cholesterol, either alone or in conjunction with other drugs conventionally used for the treatment of hypercholesterolemia.

4 Claims, 0 Drawing figures

Exemplary Claim Number: 1

---------- KWIC ---------

Brief Summary Text - BSTX (6):

The biosynthetic pathway which leads to cholesterol synthesis is tightly regulated in normal cells. From the starting product 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA), the pathway produces metabolic products which include sterols and isoprenoid products which are essential for cell function. The primary rate limiting enzyme in the pathway is HMG CoA reductase. Its activity is regulated at the level of transcription, translation, degradation and a switch from inactive to an active form. Regulation can occur by both sterol and non-sterol products of the pathway.

Brief Summary Text - BSTX (9):

U.S. Pat. No. 5,026,554 describes the squalene synthase inhibitor zaragozic acid. Billet, et al (1988) Journal of Medicinal Chemistry 31:1869 describes isoprenoid phosphinates that function as squalene synthase inhibitors in vitro, but are not effective in vivo. U.S. Pat. No. 4,871,720 describes pyrophosphinates used in the treatment of bone disorders, which function as squalene synthase inhibitors.

Brief Summary Text - BSTX (11):

U.S. Pat. No. 5,025,003 describes a number of isoprenoid derivatives

stated to be useful for inhibiting cholesterol biosynthesis. The compounds found to be effective require a phosphate moiety and a salt of a carboxylic acid. European patent No. 356,866 describes the use of farnesyloxyphosphonylmethylphosphonates and analogs thereof as inhibitors of squalene synthetase.

Detailed Description Text - DETX (15):

Farnesyl acetate and ethyl farnesyl ether stimulate a reduction in HMGal activity--In an attempt to identify tile non-sterol metabolite(s) that triggers the down regulation of HMG-CoA reductase, five commercially available isoprenoid compounds were tested for their effects on HMGal activity in CHO-HMGal cells. Each of tile compounds tested closely resembled one of tile naturally occurring isoprenoid pyrophosphates, except that the pyrophosphate group had been replaced by either a hydroxyl or an ethyl ester group. The compounds tested included: 3-methyl-2-buten-1-ol, the alcohol of the five carbon isoprenoid-dimethylallyl pyrophosphate (a naturally occurring isomer of isopentenyl pyrophosphate); geraniol and geranyl acetate, the alcohol and the ethyl ester of the ten carbon isoprenoid-geranyl pyrophosphate; and farnesol and farnesyl acetate, the alcohol and the ethyl ester of the fifteen carbon isoprenoid-farnesyl pyrophosphate. Of the five isoprenoid compounds tested, only farnesyl acetate stimulated a reduction in HMGal activity at concentrations below the levels that were toxic to the CHO-HMGal cells.

Detailed Description Text - DETX (16):

To confirm that the reduction in HMGal activity was the result of a regulatory function specifically targeting the membrane domain of HMGal, the effects of farnesyl acetate on .beta.-galactosidase activity were measured in vitro for both CHO-HMGal cells and CHO-Gal cells. The CHO-Gal cells had been stably transfected with a construct identical to the HMGal construct (using the same vector, promoter, and 5' and 3' flanking regions) except that the construct in the CHO-Gal cells codes for a soluble .beta.-galactosidase protein instead of the HMGal fusion protein with the membrane domain of HMG-CoA reductase. In this experiment, the CHO-Gal cells served to control for any artifacts farnesyl acetate might cause in the .beta.-galactosidase assay or any nonspecific effects farnesyl acetate might have on the rates of protein synthesis or degradation.

Detailed Description Text - DETX (26):

Farnesyl acetate and ethyl .farnesyl ether directly inhibit cholesterol biosynthesis After establishing that the farnesyl compounds reduce the synthesis and increase the degradation of HMG-CoA reductase, we attempted to quantify the effect that these changes would have on cholesterol biosynthesis. To allow the cells to reach steady-state levels of HMG-CoA reductase in the presence of the farnesol or sterol compounds, CHO-HMGal cells were incubated for 24 hours with either farnesyl acetate, ethyl farnesyl ether, a mixture of 25-hydroxycholesterol and cholesterol, or no additives. Subsequently, the cells were labeled for 1,2, and 4 hours with [.sup.3 H]acetate and harvested. The non-saponifiable lipid fraction from each cell type was extracted and separated by TLC. The radioactivity that co-migrated with cholesterol was measured and normalized for the amount of cellular protein. For cells treated with a mixture of 25-hydroxycholesterol and cholesterol, there was 98% less radioactive material co-migrating with cholesterol than the cells that were incubated with no additives. Previous studies have demonstrated that exogenous sterols are able to reduce 95% of cholesterol biosynthesis by down regulating enzymes in the isoprenoid metabolic pathway, primarily HMG-CoA reductase. In this study, cells treated with the farnesyl compounds exhibited a substantial

reduction in the incorporation of [.sup.3 H]acetate into cholesterol as well. Cells treated with farnesyl acetate for 24 hours showed a 80% reduction in [.sup. 3 H]acetate incorporation into cholesterol, while cells treated with ethyl farnesyl ether for 24 hours showed a 95% reduction.

Detailed Description Text - DETX (27):

Farnesyl acetate and ethyl farnesyl ether could have reduced incorporation by down regulating HMG-CoA reductase, as 25-hydroxycholesterol does, or by directly inhibiting the enzymes that synthesize cholesterol. In a number of previous studies, some of the farnesyl compounds tested have demonstrated a potent competitive inhibition of squalene synthase, the enzyme that catalyzes the first committed step in sterol branch of the isoprenoid biosynthetic pathway. In addition, one study found that some of the farnesyl compounds tested function as general inhibitors of microsomal enzymes.

Detailed Description Text - DETX (34):

It is evident from these results that farnesyl acetate and farnesyl ethyl ether are effective in stimulating post-transcriptional down-regulation of HMG-CoA reductase, the rate limiting enzyme in the synthesis of cholesterols and isoprenoids.

Other Reference Publication - OREF (4):

Biller et al., "Isoprenoid (Phosphinylmethyl)phosphonates as Inhibitors of Squalene Synthetase", Journal of Medicinal Chemistry, 31(10):1869-1871 (1988).

US-PAT-NO: 5322699

DOCUMENT-IDENTIFIER: US 5322699 A

TITLE: Leukocyte-derived CR3 modulator, integrin modulating factor-1 (IMF-1)

DATE-ISSUED: June 21, 1994

INVENTOR-INFORMATION:

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APPL-NO: 07/ 650062

DATE FILED: February 4, 1991

US-CL-CURRENT: 424/534, 424/115 , 435/7.24 , 530/395

ABSTRACT:

The invention is concerned with the discovery, isolation and purification of an agent or factor named herein the CR3 modulator or CMF-1 that is synthesized by polymorphonuclear leukocytes (PMN) in response to agonists which enhance CD18 activity. The CR3 modulator binds to CD18 and activates its adhesion-promoting ability. The CR3 modulator appears to be transiently produced by PMN as an acidic amphiphilic lipid. The CR3 modulator can be assayed by adding dilutions of a CR3 modulator -containing solution to resting PMN and observing the ability of CR3 to mediate binding of erythrocytes coated with C3bi (EC3bi). The isolation of the CR3 modulator, its biological and physical properties and diagnostic and therapeutic uses are described.

16 Claims, 17 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 17

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Brief Summary Text - BSTX (18):
(D) It appears to derive from a biosynthetic product of mevalonate synthesis and is possibly isoprenoid in structure.

Brief Summary Text - BSTX (37):
The method of inhibiting the synthesis of the CR3 modulator contemplates blocking the reaction/interaction between PMN or any active fragments thereof and the promoters of CR3 modulator synthesis. Accordingly, this embodiment of the method is directed toward the inhibition of agents that serve as CR3 modulator synthesis promoters, such as the agonists that react with receptors on polymorphonuclear leukocytes, intracellular signaling agents that initiate CR3 modulator synthesis, enzymes that synthesize the CR3 modulator, and agents that promote or participate in the synthesis or production of molecules that serve as metabolic precursors to the CR3 modulator. The method therefore

comprises introducing to a medium or other sample or substrate, antagonists or antibodies to the aforementioned agents, in amounts effective to block or inhibit the synthesis of the CR3 modulator. Representative agents that promote CR3 modulator synthesis include phorbol myristate acetate (PMA); platelet activating factor (PAF); tumor necrosis factor (TNF); formyl-NorLeu-Leu-Phe (fNLLP); interleukin-8 (IL-8) and C5a. Representative intracellular signaling agents are selected from protein kinase C, cGMP, G-proteins and mixtures. Suitable antagonists/antibodies to the promoters of CR3 modulator synthesis may be determined by appropriate assays.

Detailed Description Text - DETX (19):
(D) It appears to derive from a biosynthetic product of mevalonate synthesis and is possibly isoprenoid in structure.

Detailed Description Text - DETX (47):
In addition to the therapeutic methods that are based on the administration of an antagonist to the CR3 modulator, the invention extends to the achievement of the same therapeutic objectives by blocking the synthesis of the CR3 modulator. Thus, and as discussed earlier herein, the method of the present invention comprises the administration of antagonists, antibodies, or other agents that are able to block the action of CR3 modulator synthesis promoters. CR3 modulator synthesis promoters are meant to include, but are not limited to, agents that block or antagonize the interaction of agonists with receptors on PMN, agents that block or antagonize the production of intracellular signals that initiate CR3 modulator synthesis, agents that block or antagonize the enzymes which synthesize the CMF modulator, and agents that block or antagonize the production of molecules which serve as metabolic precursors of CR3 modulator. Representative agents that promote CR3 modulator synthesis include phorbol myristate acetate (PMA); platelet activating factor (PAF); tumor necrosis factor (TNF); formyl-NorLeu-Leu-Phe (fNLLP); interleukin-8 (IL-8) and C5a. Representative intracellular signaling agents are selected from protein kinase C, cGMP, G-proteins and mixtures. Suitable antagonists/antibodies to the promoters of CR3 modulator synthesis may be determined by appropriate assays; and the practice of the method includes the determination and/or development of appropriate antagonists or antibodies that may be formulated and administered in accordance with an acceptable therapeutic protocol.

Detailed Description Text - DETX (130):
Classes of compounds with physical properties similar to those of CMF-1 include glycerol- or sphingosine-based lipids, eicosanoids, other fatty acids, isoprenoid lipids and proteins or peptides with a hydrophobic tail. All but isoprenoids were ruled out by subjecting CMF-1 to treatments that destroy particular classes. The details of the treatments are outlined in Table 9 and summarized below and in FIG. 15.

Detailed Description Text - DETX (132):
In addition, reduction with sodium borohydride, oxidation with hydrogen peroxide, and periodate oxidation of vicinal hydroxyls all had no effect on CMF-1 activity. Of the original categories of candidate structures for CMF-1, the only ones that would remain unaffected by these treatments are isoprenoids and prostaglandins or fatty acids. A protease resistant peptide with a lipid tail is not a good possibility because CMF-1 does not absorb at 214 nm, the wavelength of absorption of the peptide bond. Prostaglandins and fatty acids are unlikely considering CMF-1 activity partitions to aqueous phase rather than the organic in a Folch partition, and since the former contain conjugated

double bond systems, ruled out by the spectrograph. This leaves an isoprenoid structure as the most likely candidate for CMF-1.

Detailed Description Text - DETX (133):
One chemical treatment that was found to destroy CMF-1 activity is ozonolysis (Table 10). Ozone (2ppm) is generated by passage of a stream of oxygen over an ultraviolet light source and bubbled into a solution of CMF-1 in ethyl acetate for 15 minutes. This treatment adds oxygen across carbon-carbon double bonds and results in double bond cleavage, forming two aldehyde molecules. Isoprenoids have double bonds, so cleavage of CMF-1 by ozone is consistent with an isoprenoid structure.

Detailed Description Text - DETX (140):
To address the possibility that CMF-1 is an isoprenoid, it was attempted to block production of CMF-1 in response to agonists by blocking the rate limiting enzyme in isoprenoid synthesis, HMGCoA reductase. A competitive inhibitor of the enzyme, lovastatin (40 ng/ml), was added to the cells to block enzyme activity. Then, to deplete the cells of any CMF-1 precursors that had passed this early step in the synthesis pathway, C5a (10.sup.-8 M) was added. C5a transiently activates cells without leaving them refractory to subsequent stimulation by PMA (11). Lovastatin-treated cells, briefly pulsed with C5a, were then activated with PMA to see if PMA could cause production of CMF-1 despite the block in isoprenoid production.

Claims Text - CLTX (2):
2. The CR3 modulator of claim 1 wherein said CR3 modulator derives from a biosynthetic product of mevalonate synthesis and possesses an isoprenoid structure.